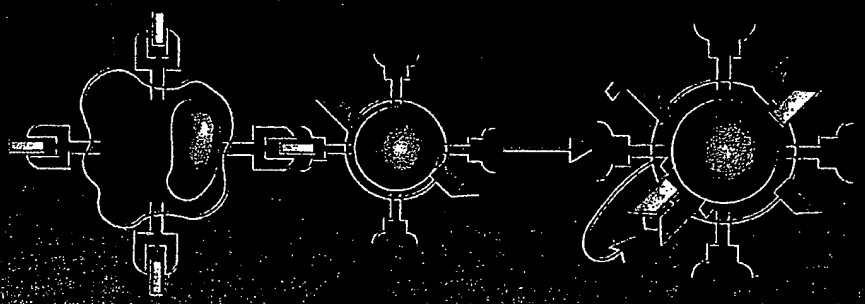
Exhibit 2

CELLULAR AND MOLECULAR IMMUNOLOGY



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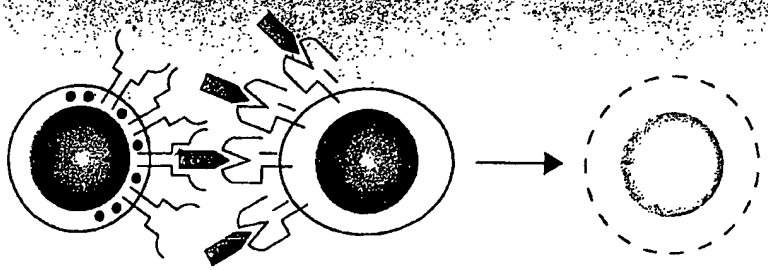

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CHAPTER FIFTEEN

IMMUNITY TO MICROBES

The principal physiologic function of the immune system is to protect the host against pathogenic microbes. Resistance to infections formed the basis for the original identification of acquired immunity. As our understanding of specific immune responses has increased, we are better able to explain the mechanisms of anti-microbial immunity. Throughout this book we have mentioned examples of specific immune responses to particular microbes, largely to illustrate the physiologic relevance of various aspects of lymphocyte function. In this chapter, we discuss in more detail the main features of immunity to different types of pathogenic microorganisms.

The evolution of an infectious disease in an individual involves a sequence of interactions between the microbe and the host. These include entry of the microbe, invasion and colonization of host tissues, evasion from host immunity, and tissue injury or functional impairment. Some microbes produce disease by liberating toxins, even without extensive colonization of host tissues. Many features of microorganisms determine their virulence, and many diverse mechanisms contribute to the pathogenesis of infectious diseases. These are largely beyond the scope of this book and will not be discussed in detail. Rather, our discussion will focus on the host immune response to pathogenic microorganisms. There are several important general features of immunity to microbes:

1. *Defense against microbes is mediated by both natural and acquired immunity.* Microbial infections provide clear demonstrations of the role of specific immunity in enhancing the protective mechanisms of natural immunity and in directing these mechanisms to sites where they are needed.

2. *Different types of microbes stimulate distinct lymphocyte responses and effector mechanisms.* Because microbes differ greatly in patterns of host invasion and colonization and in their immunogenicity, their elimination requires diverse effector systems. Studies in humans and experimental models have led to reports of virtually every type of immune response to infections by different classes of microorganisms. Our subsequent discussions will attempt to highlight the principal mechanisms of specific immunity against different bacteria, viruses and parasites.

3. *The survival and pathogenicity of microbes in a host are critically influenced by their ability to evade or resist protective immunity.* Microorganisms have developed a variety of strategies for surviving in the face of powerful immunologic defenses.

4. *Tissue injury and disease consequent to infections may be caused by the host response to the microbe and its products rather than by the microbe itself.* Immunity, like many other homeostatic mechanisms, is necessary for host survival but also has the potential of causing injury to the host.

This chapter considers four types of pathogenic microorganisms: (1) extracellular bacteria, (2) intracellular bacteria, (3) viruses, and (4) parasites. In each group, selected examples will be used to highlight key points. As we shall see, these four groups of microbes illustrate the diversity of antimicrobial immunity and the physiologic significance of many of the responses and effector functions of lymphocytes discussed in earlier chapters.

IMMUNITY TO EXTRACELLULAR BACTERIA

Extracellular bacteria are capable of replicating outside host cells, e.g., in the circulation, in extracellular connective tissues, and in various tissue spaces such as the airways and intestinal lumens. These bacteria include gram-positive pus-forming, or pyogenic, cocci (*Staphylococcus, Streptococcus*), gram-negative cocci (meningococcus and gonococcus, two species of *Neisseria*), many gram-negative bacilli (including enteric organisms such as *Escherichia coli*) and some gram-positive bacilli (particularly anaerobes such as the *Clostridium* species).

Extracellular bacteria cause disease by two principal mechanisms. First, they induce inflammation, which results in tissue destruction at the site of infection. Pyogenic cocci are responsible for a large number of suppurative infections in humans. Second, many of these bacteria produce **toxins**, which have diverse pathologic effects. Such toxins may be **endotoxins**, which are components of bacterial cell walls, or **exotoxins**, which are actively secreted by the bacteria. The endotoxin of gram-negative bacteria, also called **lipopolysaccharide** (LPS), has been mentioned in earlier chapters as a potent stimulator of cytokine production, an adjuvant, and a polyclonal activator of B cells. Many exotoxins are primarily cytotoxic, and they kill cells by poorly defined mechanisms. There are also many other examples of exotoxins whose mode of action is known in precise detail. For instance, diphtheria toxin inhibits protein synthesis, by enzymatically modifying and thereby blocking the function of elongation factor-2, which is necessary for the synthesis of all polypeptides. Cholera toxin synthesis stimulates cyclic adenosine monophosphate (cAMP) production in intestinal epithelial cells, leading to active chloride secretion, water loss, and intractable diarrhea. Tetanus toxin is a neurotoxin that binds to motor end plates at neuromuscular junctions and causes persistent muscle contraction, which can be fatal if it affects the muscles involved in breathing. Clostridial toxins cause extensive tissue necrosis and lead to gas gangrene. *Immune responses against extracellular bacteria are aimed at eliminating the bacteria and at neutralizing the effects of their toxins.*

Natural Immunity to Extracellular Bacteria

Because extracellular microbes are rapidly killed by the microbicidal mechanisms of phagocytes, a principal mechanism of natural immunity to these microbes is phagocytosis by neutrophils, monocytes, and tissue macrophages. The resistance of bacteria to phagocytosis and digestion within macrophages is an

important determinant of virulence. Activation of the complement system, in the absence of antibody, also plays an important role in the elimination of these bacteria. Gram-positive bacteria contain a peptidoglycan in their cell walls that activates the alternative pathway of complement by promoting the formation of the alternative pathway C3 convertase (see Chapter 13). LPS in the cell walls of gram-negative bacteria was one of the first agents shown to activate the alternative complement pathway, in the absence of antibody. LPS may provide a site for C3b deposition where the bound C3b is protected from inactivation by factors H and I. It may also directly bind C1q and activate the classical pathway of complement, without a requirement for antibody. One result of complement activation is the generation of C3b, which opsonizes bacteria and enhances phagocytosis. In addition, the membrane attack complex (MAC) lyses bacteria, and complement by-products participate in inflammatory responses by recruiting and activating leukocytes.

Endotoxins, such as LPS, stimulate the production of cytokines by macrophages and by other cells, e.g. vascular endothelium. These cytokines include tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), and low molecular weight inflammatory cytokines, which are members of the interleukin-8 (IL-8) family. The structure and functional effects of these cytokines have been discussed in Chapter 11. The principal physiologic functions of macrophage-derived cytokines are to stimulate nonspecific inflammation and to enhance the activation of specific lymphocytes by bacterial antigens. Thus, cytokines induce the adhesion of neutrophils and monocytes to vascular endothelium at sites of infection, which is followed by migration, local accumulation, and activation of the inflammatory cells. These inflammatory cells serve to eliminate the bacteria; injury to adjacent normal tissues is a pathologic side effect of these defense mechanisms. Cytokines also induce fever and stimulate the synthesis of acute phase proteins, two responses whose physiologic roles are not well understood (see Chapter 11, Box 11-2). Many of the same cytokines function as costimulators of T and B lymphocytes, providing amplification mechanisms for specific immunity.

Large amounts of cytokines or their uncontrolled production can be harmful and are responsible for some of the clinicopathologic manifestations of infections by extracellular bacteria. The most severe cytokine-induced pathologic consequence of infection by gram-negative bacteria is progressive disseminated intravascular coagulation (DIC) and vascular collapse, also called "septic shock" or "endotoxin shock." As discussed in Chapter 11, TNF is the principal mediator of endotoxin shock.

Specific Immune Responses to Extracellular Bacteria

Humoral immunity is the principal protective specific immune response against extracellular bacteria. Some of the most immunogenic components of the cell walls and capsules of these microbes are polysaccharides, which are prototypical thymus-independent antigens. Such antigens directly stimulate B cells, giving rise to strong specific IgM responses. In addition, other immunoglobulin (Ig) isotypes may be produced, probably as a result of the production of cytokines that promote heavy chain isotype switching (see Chapter 9). The best documented example of heavy chain class switching induced by a T cell-independent antigen is the humoral immune response to pneumococcal capsular polysaccharides in humans, which is dominated by IgG2 antibodies.

The principal T cell response to extracellular bacteria consists of $CD4^+$ T cells responding to protein antigens in association with class II major histocompatibility complex (MHC) molecules. As discussed in Chapter 6, extracellular microbes and soluble antigens are phagocytosed by antigen presenting cells (APCs), the antigens are processed, and fragments of the processed proteins preferentially associate with class II MHC molecules. It is not known whether macrophages, B cells, or other cell types are the most important APCs for such bacterial protein antigens *in vivo*. $CD4^+$ T cells function as helper cells to stimulate antibody production and to activate the phagocytic and microbicidal functions of macrophages.

Recently, it has been observed that some bacterial toxins stimulate large numbers of $CD4^+$ T cells. Any one of these toxins can stimulate all the T cells in an individual that express a particular set or family of V_β T cell receptor genes. Such toxins have been called **super-antigens** (Box 15-1). Their importance lies in their ability to activate many T cells, resulting in large amounts of cytokine production and clinicopathologic abnormalities that may be similar in some respects to endotoxin shock.

Both IgM and IgG antibodies against bacterial surface antigens and toxins stimulate three types of **effector mechanisms:**

1. *IgG antibodies opsonize bacteria and enhance phagocytosis* by binding to Fcγ receptors on monocytes, macrophages, and neutrophils (see Chapter 3). Both IgM and IgG antibodies activate complement, generating C3b and iC3b, which bind to specific type 1 and type 3 complement receptors, respectively, and further promote phagocytosis. Individuals deficient in C3 are extremely susceptible to pyogenic infections.

2. *Both IgG and IgM antibodies neutralize bacterial toxins,* prevent their binding to target cells, and promote their clearance by phagocytosis. Passive immunization against tetanus toxin by injection of antibody is a potentially life-saving treatment in acute tetanus infections. IgA antibody present in various secretions, e.g., in the gastrointestinal and respiratory tracts, is important for neutralizing the toxins of bacteria in these organs and for preventing colonization of extraluminal tissues.

3. *Both IgM and IgG antibodies activate the complement system,* leading to the production of the microbicidal MAC and the liberation of by-products that

BOX 15–1. BACTERIAL 'SUPER-ANTIGENS'

Staphylococcal enterotoxins (SEs) are exotoxins produced by the gram-positive bacterium *Staphylococcus aureus* and consist of five serologically distinct groups of proteins, SEA, SEB, SEC, SED, and SEE. These toxins are the most common cause of food poisoning in humans. A related toxin, TSST, causes a disease called the toxic shock syndrome (TSS), which is associated with tampon use. Pyrogenic exotoxins of streptococci and exotoxins produced by mycoplasma may be structurally and functionally related to these enterotoxins.

The immune response to staphylococcal enterotoxins and related proteins has a number of features that make these bacterial products quite unique in terms of biologic and pathologic effects:

1. *Staphylococcal enterotoxins are among the most potent naturally occurring T cell mitogens known.* They are capable of stimulating the proliferation of normal T lymphocytes at concentrations of 10^{-9} M or less. The observed mitogenic effect of staphylococcal protein A preparations is actually due to contaminating enterotoxins. As many as one in five normal T cells in mouse lymphoid tissue or human peripheral blood may respond to a particular enterotoxin.

2. *The T cells that respond to each enterotoxin express V_β genes from a particular V_β family in their antigen receptors.* Responsiveness is apparently not related to other components of the T cell receptor (TCR), i.e., V_α, J, or D segments. Different enterotoxins stimulate T cells expressing V_β genes from different families (see table). Because T cells expressing only certain TCRs recognize and respond to each enterotoxin, these proteins are called antigens and not polyclonal mitogens. However, since the frequency of enterotoxin-responsive T cells is much higher than the frequency of T cells specific for conventional protein antigens, the enterotoxins have been named "super-antigens."

3. *Responses to staphylococcal enterotoxins require antigen-presenting cells that express class II MHC molecules.* Depletion of class II–bearing cells or addition of anti–class II antibody inhibits the T cell proliferative response to these toxins.

4. *Staphylococcal enterotoxins directly bind to class II MHC molecules on accessory cells.* This complex is then recognized by T cells expressing antigen receptors with a particular V_β. It is likely that enterotoxins do not need to be processed like other protein antigens before they bind to MHC molecules, and they do not associate with MHC molecules in the peptide-binding cleft. The same enterotoxin binds to class II molecules of different alleles, indicating that the polymorphism of the MHC does not influence presentation of these antigens.

The remarkably high frequency of staphylococcal enterotoxin-responding CD4+ T cells has several functional implications. Acutely, exposure to high concentrations of enterotoxins leads to systemic reactions like fever, DIC, and cardiovascular shock. These abnormalities are probably mediated by cytokines, such as TNF, produced directly by the T cells or by macrophages that are activated by the T cells. This is the likely pathogenesis of the toxic shock syndrome, which is characterized by shock, skin exfoliation, conjunctivitis, and severe gastrointestinal upset, and can progress to renal and pulmonary failure and death. More prolonged administration of enterotoxins to mice results in wasting, thymic atrophy, and profound immunodeficiency, also probably secondary to chronic high levels of cytokine (e.g., TNF) production.

Staphylococcal enterotoxins are proving to be useful tools for analyzing T lymphocyte development. Administration of SEB to neonatal mice leads to intrathymic deletion of all T cells expressing $V_\beta3$ and $V_\beta8$ TCR genes. This mimics the postulated self antigen–induced clonal deletion of self-reactive T cells during thymic maturation.

The concept of super-antigens may extend beyond bacterial products. Mice express polymorphic genes called *Mls* (for "minor lymphocyte stimulating" antigen) that are not linked to the MHC complex and are detected by the ability to stimulate strong mixed lymphocyte reactions. Thus, T cells from mice expressing one *Mls* allele proliferate in response to stimulator cells expressing a different *Mls* allele, even if the two strains are identical at all MHC loci. The *Mls* gene product functions as a super-antigen, because any allelic difference stimulates virtually all T cells expressing a V_β gene from a particular V_β family. As expected, in mice expressing one *Mls* allele, all T cells whose antigen receptors contain V_β reactive with that allele are deleted during thymic development.

V_β Expression in Responding T Cells

Enterotoxin	Mice	Humans
SEB	V_β3, 7, 8.1–8.3, 17	V_β3, 12, 14, 15, 17, 20
SEC 2	V_β3, 8.2, 10, 17	V_β12, 13.1, 13.2, 14, 15, 17, 20
SEE	V_β11, 15, 17	V_β5.1, 6.1–6.3, 8, 18
TSST	V_β3, 15, 17	V_β2

Abbreviations: SE, staphylococcal enterotoxin, TSST, toxic shock syndrome toxin.

are mediators of acute inflammation. It is likely, however, that the lytic function of the MAC is important for the elimination of only some microbes. As we mentioned in Chapter 13, deficiencies of the late components of complement, C5 to C9, which are involved in the formation of the complex, are associated with increased susceptibility to *Neisseria* but not other bacterial infections.

As mentioned earlier, acute inflammation and endotoxin shock are two of the injurious consequences of defense against extracellular bacteria. A late complication of the humoral immune response to bacterial infections may be the generation of disease-producing antibodies. The best-defined examples are two sequelae of streptococcal infections of the throat or skin, which are manifested weeks or even months after the infections are controlled. In **rheumatic fever**, pharyngeal infection with some serologic types of β-hemolytic streptococci leads to the production of antibodies against a bacterial cell wall protein (M protein). Some of these antibodies cross-react with

myocardial sarcolemmal proteins and myosin, leading to antibody deposition in the heart and subsequent inflammation (carditis). In **post-streptococcal glomerulonephritis,** infection of the skin or throat with other serotypes of β-hemolytic streptococci leads to the formation of immune complexes of bacterial antigen and specific antibody. The complexes deposit in kidney glomeruli and produce nephritis. It is worth noting that thorough antibiotic therapy is recommended for "sore throats" caused by β-hemolytic streptococci, not because of the severity of the pharyngitis but to prevent the later development of rheumatic fever.

Other bacterial infections may lead to different sequelae. The polyclonal lymphocyte activation induced by bacterial endotoxins and super-antigens may also contribute to the development of autoimmunity. Such bacterial infections may lead to the stimulation of many lymphocytes, among which are self-reactive clones that are normally anergic to stimulation by self antigens. This concept, and other possible links between infections and autoimmune diseases, are discussed more fully in Chapter 18.

Evasion of Immune Mechanisms by Extracellular Bacteria

The virulence of extracellular bacteria has been linked to a number of mechanisms that favor tissue invasion and colonization. These include adhesive properties of bacterial surface proteins, anti-phagocytic mechanisms, and inhibition of complement or inactivation of complement products. For instance, the capsules of many gram-positive and gram-negative bacteria contain one or more sialic acid residues that inhibit complement activation by the alternative pathway. Encapsulated bacteria also resist phagocytosis and, therefore, are much more virulent than homologous strains lacking a capsule.

One mechanism utilized by bacteria to evade specific immunity is *genetic variation of surface antigens.* Surface antigens of many bacteria, such as gonococci and *E. coli,* are contained in the pili, which are the structures primarily involved in bacterial adhesion to host cells. The major antigen of the pili is a protein of approximately 35 kilodaltons (kD) called pilin. The pilin genes of gonococci consist of one or two expression loci. In addition, there are ten to 20 silent loci, each containing six coding sequences, called "minicassettes." Antigenic variation results from a high rate of conversion between silent and expression loci. A gene conversion event replaces the minicassette on the expression locus with a duplicate of one of the minicassettes from one of the silent loci (Fig. 15-1). From ten silent loci, each with six minicassettes, it is possible to create 10^6 combinations whose protein products are antigenically distinct. This mechanism helps the bacteria to escape specific antibody attack, although its principal significance for the bacteria may be to select for pili that are more adherent for host cells so that the bacteria are more virulent.

IMMUNITY TO INTRACELLULAR BACTERIA

A number of bacteria, and all viruses, survive and replicate within host cells. Among the bacteria, some of the most pathogenic are ones that are resistant to degradation in macrophages and are therefore capable of surviving within phagocytes. Two of the best known examples are mycobacteria and *Listeria monocytogenes.* Since these microbes are able to find a niche where they are inaccessible to circulating antibodies, their elimination requires immune mechanisms that are very different from the mechanisms of defense against extracellular bacteria. Many fungi are also capable of surviving within host cells, and defense against them is mediated by mechanisms similar to those against intracellular bacteria.

Natural Immunity to Intracellular Bacteria

The principal mechanism of natural immunity against intracellular microbes is phagocytosis. However, pathogenic intracellular bacteria are relatively resistant to degradation within mononuclear phagocytes. It is, therefore, not surprising that usually *natural immunity is quite ineffective in controlling colonization by and spread of these microorganisms.* Resistance to phagocytosis is also the reason why such bacteria tend to cause chronic infections that may last for years, often recur or recrudesce after apparent cures, and are difficult to eradicate.

Specific Immune Responses to Intracellular Bacteria

The major protective immune response against intracellular bacteria is cell-mediated immunity. Cell-mediated immunity was first identified by George Mackaness in the 1950s as protection against the intracellular bacterium *L. monocytogenes.* This form of immunity could be adoptively transferred to naive animals with lymphoid cells but not with serum from infected or immunized animals. We now know that the specificity of cell-mediated immunity is due to T lymphocytes, but the effector function of bacterial elimination is mediated by macrophages that are activated by T cell-derived cytokines, particularly γ-interferon (IFN-γ) (Fig. 15-2). The immune response to such bacteria is analogous to delayed type hypersensitivity (DTH) reactions to soluble protein antigens (see Chapter 12).

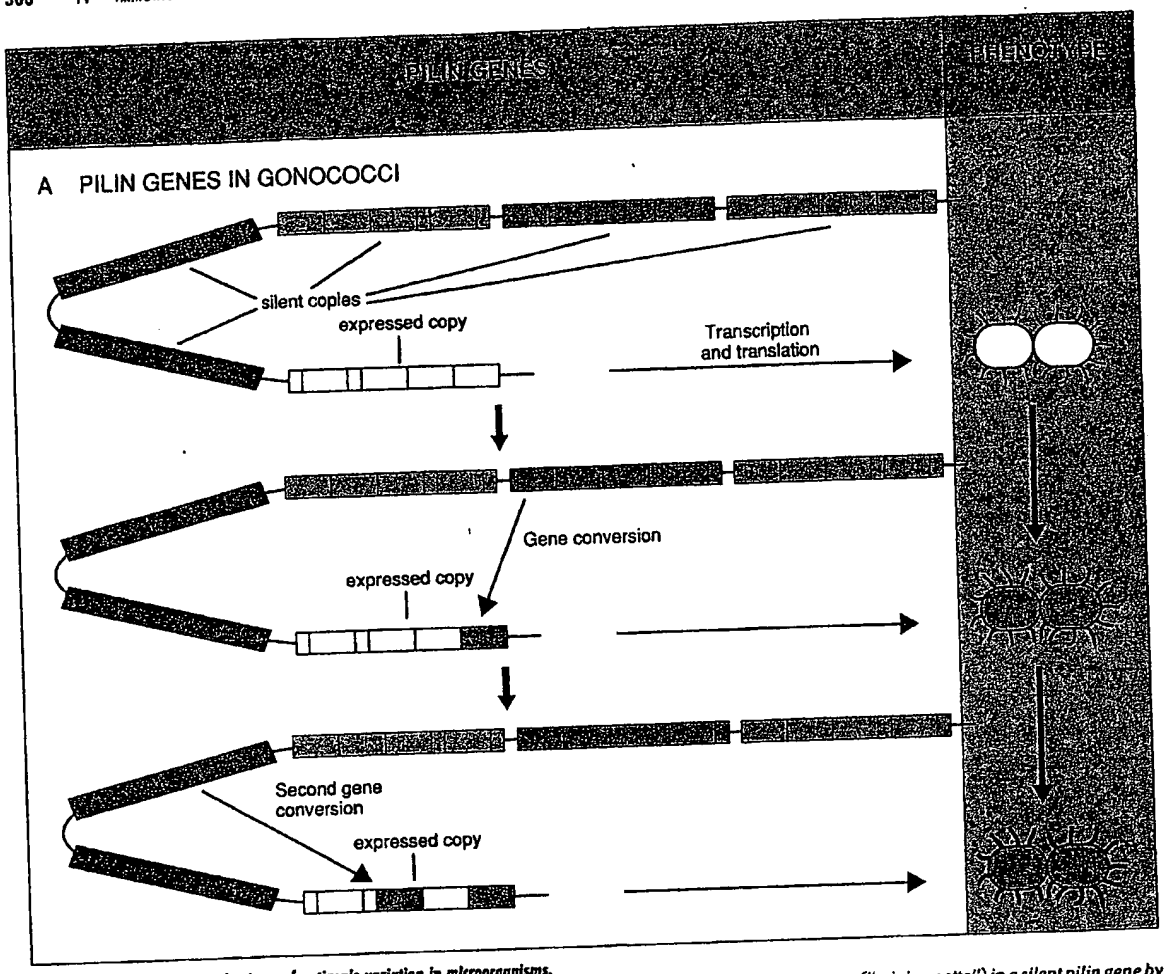


FIGURE 15-1. Genetic mechanisms of antigenic variation in microorganisms.
A. *In gonococci, a segment of the expressed pilin gene may be replaced by nucleotides from a DNA segment ("minicassette") in a silent pilin gene by a process of gene conversion, generating a new pilin gene and expressed protein. (Sizes of DNA segments are not to scale, and only five silent copies of the pilin gene are shown.)*

The protein antigens of intracellular bacteria stimulate strong T cell responses. Many such microbes contain cell wall constituents that activate macrophages directly and therefore function as adjuvants. An example of such an adjuvant is muramyl dipeptide, present in the cell walls of mycobacteria. Adoptive transfer experiments have shown that both $CD4^+$ and $CD8^+$ T cells contribute to protective immunity against intracellular bacteria. These T cell subsets may recognize and respond to different types of antigens. For instance, in mycobacterial infections, antigens such as the purified protein derivative (PPD) stimulate $CD4^+$ T cells and these cells mediate DTH reactions to skin challenge with PPD in previously infected persons. $CD8^+$ T cells may recognize bacterial antigens that are produced in infected cells. The relative contribution of $CD4^+$ and $CD8^+$ T cells to immunity against different intracellular bacteria is not known. However, the principal function of both T cell subsets in cell-mediated immunity is the production of cytokines, particularly IFN-γ.

The consequence of IFN-γ production by specific T cells is the activation of macrophages, including ones that are infected. IFN-γ stimulates phagocytic and degradative functions of macrophages, leading to enhanced bacterial killing. As a result of macrophage activation, the number of viable bacteria may be drastically reduced and often completely eradicated. However, intracellular microbes have evolved to resist phagocytes and often persist for long periods, even in individuals with effective cell-mediated immunity. Persistent organisms provide chronic antigenic stimulation. This may lead to local collections of activated macrophages, called **granulomas** (Chapter 12, Fig. 12-6), surrounding the microbes and preventing their spread. The histologic hallmark of many mycobacterial and fungal infections is granulomatous inflammation. This type of inflammation is associated with tissue necrosis and extensive fibrosis, leading to severe functional impairment. *Thus, the host immune*

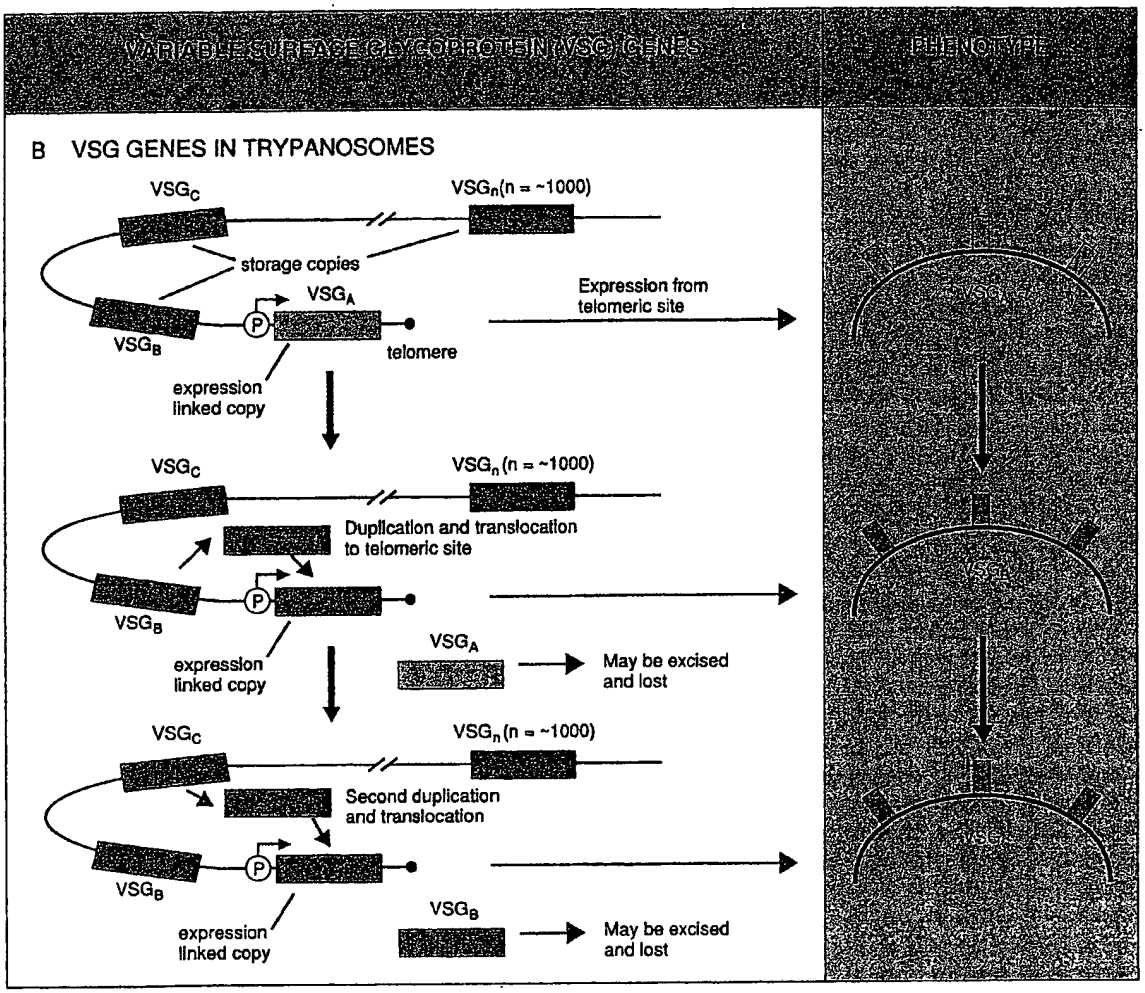


FIGURE 15–1. Continued
B. *In trypanosomes, the expressed variable surface glycoprotein (VSG) is encoded by a gene located close to the telomere. Another VSG gene may be duplicated and translocated to this telomeric expression site, generating a new VSG. The fate of the previously expressed VSG gene is not known, but it may be excised and lost (as shown). (P), promoter.*

response is the principal cause of tissue injury and disease in infections by some intracellular bacteria. This is most clearly illustrated in mycobacterial infections. Mycobacteria do not produce any known toxins or enzymes that directly injure infected tissues. The first exposure to *Mycobacterium tuberculosis* induces a local cellular inflammation, and the bacteria initially proliferate within phagocytes. They may then die or lie dormant. At the same time, the infected individual develops specific T cell immunity. After immunity has developed, severe granulomatous reactions can occur at sites of bacterial persistence or upon subsequent exposures to the bacteria. Thus, both protective immunity and the hypersensitivity reaction that produces tissue injury are manifestations of the same type of specific immune response.

Differences among individuals in the patterns of immune responses to intracellular microbes are important determinants of disease progression and clinical outcome. An example of this is leprosy, caused by *Mycobacterium leprae.* There are two polar forms of the disease, although many patients fall in less clear intermediate groups. In lepromatous leprosy, patients have high specific antibody titers but weak cell-mediated responses to *M. leprae* antigens. Mycobacteria proliferate within macrophages, presumably because of poor cell-mediated immunity. The bacterial growth and macrophage activation result in destructive lesions of skin and underlying bones. In contrast, patients with tuberculoid leprosy have strong cell-mediated immunity but low antibody levels. This pattern of immunity is reflected in granulomas that form around nerves, giving rise to sensory peripheral nerve defects and secondary traumatic skin lesions. Such

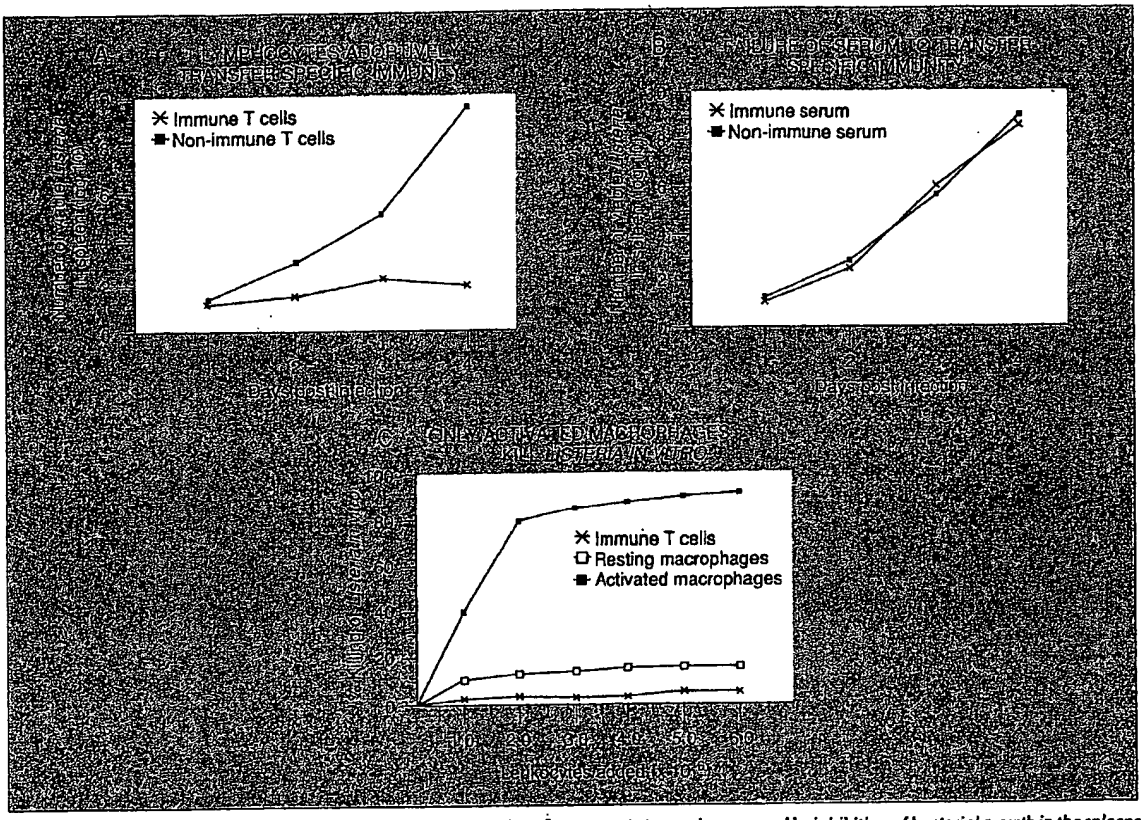


FIGURE 15–2. Cell-mediated immunity to Listeria monocytogenes. *Immunity to* L. monocytogenes *is measured by inhibition of bacterial growth in the spleens of animals inoculated with a known dose of viable bacteria. Such immunity can be transferred to normal mice by T lymphocytes* (A) *but not by serum* (B) *from syngeneic mice previously immunized with killed* Listeria monocytogenes. *However, the actual killing of bacteria* in vitro *is mediated by activated macrophages and not by T cells, even from immune animals* (C).

differences suggest that in different individuals *M. leprae* stimulates the production of cytokines that either selectively help B cells or activate macrophages. This may be due to varying levels of T cell activation, or differential expansion of distinct T cell subsets that may be analogous to the Th1 and Th2 clones described in mice (mentioned in Chapters 9 and 10). In fact, T cells from patients with lepromatous leprosy produce less IL–2 and IFN–γ in response to *M. leprae* than cells from tuberculoid leprosy patients. Furthermore, intradermal injection of IFN–γ has a beneficial effect on the skin lesions of lepromatous leprosy.

Several recent studies have shown that mycobacteria activate T cells bearing the $\gamma\delta$ form of the T cell receptor (TCR). Mice infected with *M. tuberculosis* show increased numbers of $\gamma\delta^+$ cells in draining lymph nodes. If peripheral blood lymphocytes are stimulated by *M. tuberculosis*–infected macrophages, $\gamma\delta$-expressing T cells are preferentially expanded. Furthermore, molecular cloning of mycobacterial antigens has shown that some of the immunodominant antigens are homologous to **heat shock proteins**, which are also known to activate $\gamma\delta$ T cells. Heat shock proteins are evolutionarily conserved molecules found in many prokaryotes and eukaryotes. They are induced upon exposure to different types of stress, including heat, contact with free radicals, and deprivation of oxygen, nutrients, or essential ions. Intracellular bacteria are exposed to anoxia and free radicals within phagocytes and to fever associated with infection. All these stimuli could lead to the production of heat shock proteins by the bacteria and, perhaps, by infected host cells as well. It has been postulated that the reaction of $\gamma\delta$-bearing T cells to heat shock proteins is an example of a primitive and rather nonspecific mechanism of defense against some microbes and their protein products. However, at present we do not know the significance of this rare T cell subset in immunity to mycobacteria and other intracellular microbes.

Evasion of Immune Mechanisms by Intracellular Bacteria

An important mechanism for survival of intracellular bacteria is their ability to resist elimination by phago-

cytes. Mycobacteria do this by inhibiting phagolysosome fusion, perhaps by interfering with lysosome movement. The phenolic glycolipid of *M. leprae* functions as a scavenger of reactive oxygen species. Virulent strains of *L. monocytogenes* produce a protein called hemolysin, which promotes intracellular bacterial survival, probably by forming pores in the phagosome membrane, thereby releasing bacteria into the cytoplasm and preventing their degradation in phagolysosomes. (Hemolysin may also block antigen processing by macrophages and reduce the specific T cell response.) *Legionella pneumophila* is an intracellular bacterium that is the causative organism of Legionnaire's disease. Mutants of these bacteria that lose their ability to inhibit phagolysosome fusion also lose their virulence.

IMMUNITY TO VIRUSES

Viruses are obligatory intracellular microorganisms that replicate within cells, often using the nucleic acid and protein synthetic machineries of the host. Many viruses enter host cells by binding to physiologically important, normal cell surface molecules. Three well-known examples are (1) human immunodeficiency virus-1 (HIV-1), which binds to the CD4 molecule on human T cells; (2) Epstein-Barr virus (EBV), which binds to the type 2 complement receptor on human B cells; and (3) rhinovirus, the agent of the common cold, which binds to intercellular adhesion molecule (ICAM-1) expressed on a variety of cell types, including airway epithelium.

After entering cells, viruses can cause tissue injury and disease by any of several mechanisms. Viral replication interferes with normal cellular protein synthesis and function, leading to injury to and ultimately death of the infected cell. This is one type of **cytopathic effect of viruses,** and the infection is said to be "lytic" because the infected cell is lysed. Noncytopathic viruses may cause latent infections, during which they reside in host cells and produce proteins that are foreign to the host and stimulate specific immunity. As a result, infected cells are recognized and killed by viral antigen-specific cytolytic T lymphocytes (CTLs). Released viral proteins may also stimulate DTH reactions. *In these situations, cell injury is a direct consequence of physiologic immune responses to the virus.* Relatively little is known about the pathogenic mechanisms in many other viral infections, e.g., slow virus-induced demyelination and hepatitis virus-induced liver injury. Human immunodeficiency virus is discussed in Chapter 19.

Protective immunity against viruses operates at two stages: in the *initial phase of infection,* before a virus has invaded host cells, and *after invasion into cells,* when the virus is inaccessible to antibodies and phagocytes. Furthermore, because different viruses can infect a wide variety of cell types, anti-viral immunity must be capable of acting on diverse populations of infected cells.

Natural Immunity to Viruses

There are two principal mechanisms of natural immunity against viruses:

1. *Viral infection directly stimulates the production of type I IFN by infected cells.* Type I IFN functions to inhibit viral replication. The characteristics of the cytokine-induced "anti-viral state" have been described in Chapter 11.
2. *Natural killer (NK) cells lyse a wide variety of virally infected cells.* NK cells may be one of the principal mechanisms of immunity against viruses early in the course of infection, before specific immune responses have developed (see Chapter 12). Type I IFN can enhance the ability of NK cells to lyse infected target cells.

In addition, complement activation and phagocytosis serve to eliminate viruses from extracellular sites and from the circulation.

Specific Immune Responses to Viruses

Immunity against viral infections is mediated by a combination of humoral and cellular immune mechanisms. *Specific antibodies are important in defense against viruses early in the course of infection.* Neutralizing anti-viral antibodies bind to envelope or capsid proteins and prevent viral attachment and entry into host cells. Opsonizing antibodies may enhance phagocytic clearance of viral particles. Somewhat perversely, however, opsonizing antibodies may actually enhance the invasion of Fc receptor-bearing cells by viruses; this has been postulated to be a mechanism for HIV-1 infection of mononuclear phagocytes. Secretory immunoglobulins of the IgA isotype may be important for neutralizing viruses that enter via the respiratory or intestinal tract. Induction of secretory immunity is one of the bases for oral immunization against poliomyelitis. Complement activation may also participate in antibody-mediated viral immunity, mainly by promoting phagocytosis and possibly by direct lysis of viruses with lipid envelopes.

The success of prophylactic vaccination with attenuated or killed viruses is largely related to the ability of these vaccines to stimulate specific antibody responses. The importance of humoral immunity is suggested by the observation that resistance to a particular virus, induced either by infection or vaccination, is often specific for the serologic type of the virus and seems to correlate with antibody specificity. An example of this is influenza virus, in which exposure to one serologic type does not confer resistance to other serotypes of the virus. However, several points about the role of humoral immunity in protection against viruses should be emphasized. First, antibodies are of protective value only in the early phase of viral infection, before the microorganism has gained a foothold in its sequestered location inside host cells.

Second, it has generally proved difficult to transfer anti-viral immunity to naive animals with purified antibodies. Third, the neutralizing capacity of an antibody *in vitro* usually shows little or no correlation with its protective capacity *in vivo*. Taken together, these observations suggest that antibodies are an important component of immunity to viruses but may not be sufficient for eliminating many viral infections.

The principal mechanism of specific immunity against established viral infections is CTLs. The best-defined virus-specific CTLs are $CD8^+$ cells that recognize endogenously synthesized viral antigens in association with class I MHC molecules on virtually any cell type. A smaller but detectable proportion of virus-specific CTLs in humans and mice consists of $CD4^+$ CTLs that recognize viral antigens presented in association with class II MHC molecules. $CD4^+$ CTLs can be effective only against infected cells that express class II molecules, whereas $CD8^+$ CTLs have a much broader range of cellular reactivity. The full differentiation of $CD8^+$ CTLs requires cytokines produced by $CD4^+$ helper cells, which recognize endogenously synthesized or shed viral antigens in association with class II molecules. As discussed in Chapter 12, the anti-viral effects of CTLs are due to lysis of infected cells, stimulation of intracellular enzymes that degrade viral genomes, and secretion of cytokines with interferon activity.

The importance of CTLs in the outcome of viral infections has been demonstrated in many experimental systems. Mice can be protected against influenza virus by adoptive transfer of virus-specific, class I–restricted CTLs and by cloned lines of such T cells. Interestingly, a large proportion of influenza-specific CTLs are not serotype-specific because they recognize peptides derived from internal proteins (like matrix protein and nucleoprotein) rather than the envelope proteins (hemagglutinin, neuraminidase) that determine serotype. Nevertheless, as mentioned above, actively acquired immunity to influenza virus is serotype-specific. These findings support the view that both antibodies and CTLs cooperate to protect the host against viruses—the former act to block viral binding and entry into host cells, and the latter inhibit viral replication in infected cells.

In some infections with non-cytopathic viruses, CTLs may be responsible for tissue injury. The best example is lymphocytic choriomeningitis virus (LCMV), which induces inflammation of the spinal cord meninges in mice. LCMV infects meningeal cells but does not injure them. It stimulates the development of specific CTLs that lyse meningeal cells during a physiologic attempt to eradicate the viral infection. T cell–deficient mice infected with LCMV become chronic carriers of the virus but pathologic lesions do not develop, whereas in normal mice meningitis develops. On face value, this observation appears to contradict the usual situation, in which immunodeficient individuals are more susceptible to infectious diseases than normal individuals. Hepatitis B virus infection in humans shows some similarities to murine LCMV, in that immunodeficient persons who become infected do not develop the disease, but become carriers who can transmit the infection to otherwise healthy persons. The livers of patients with acute hepatitis contain large numbers of $CD8^+$ T cells, but the antigenic specificity and MHC restriction of these cells have not been defined.

Viral infections, and immune responses to them, may be involved in producing disease in two other ways. First, a consequence of persistent infection with some viruses, such as hepatitis B, is the formation of circulating immune complexes composed of viral antigens and specific antibodies. These complexes deposit in blood vessels and lead to widespread, destructive vasculitis (see Chapter 18). Second, some viruses are known to contain amino-acid sequences that are also present in some self antigens. It has been postulated that because of this "molecular mimicry" antiviral immunity can lead to immune responses against self antigens.

Evasion of Immune Mechanisms by Viruses

The intracellular persistence of viruses is the most obvious mechanism by which they can be hidden from immune effector cells and molecules. Viruses have evolved other mechanisms for evading host immunity:

1. *Many viruses are capable of great antigenic variation,* and large numbers of serologically distinct strains of these viruses have been identified. The influenza pandemics that occurred in 1918, 1957, and 1968 were all due to different strains of the virus, and subtler variants arise more frequently. As a result, the virus becomes insusceptible to immunity generated in the population by previous infections. There are so many existing serotypes of rhinovirus that specific immunization against the common cold may not be a feasible preventive strategy. In these situations, prophylactic vaccination may have to be directed against invariant viral proteins, such as surface molecules that mediate virus binding and entry into host cells.

2. *Viruses suppress immune responses by various mechanisms.* Some viruses may infect the cells of the immune system, impairing their function and resulting in inhibition of specific immunity. The most obvious example of this is, of course, HIV-1–induced acquired immunodeficiency syndrome (AIDS). Immune suppression has been described in infections with retroviruses, Epstein-Barr virus (EBV), and numerous others, but the mechanisms are not well defined. One intriguing possibility has been suggested by the recent observation that an EBV gene is homologous to a mammalian gene that encodes a cytokine called "cytokine synthesis inhibitory factor" or "CSIF." CSIF inhibits the production of other cytokines, including IL-2 and IFN-γ. Thus, pathogenic viruses may contain or may have acquired genes whose products inhibit antiviral immune responses.

IMMUNITY TO PARASITES

In infectious disease terminology, "parasitic infection" refers to infection with animal parasites, such as protozoa, helminths, and ectoparasites (e.g., ticks and mites). Such parasites currently account for greater morbidity and mortality than any other class of infectious organisms, particularly in developing countries. It is estimated that about 30 per cent of the world's population suffers from parasitic infestations. Malaria alone affects almost 300 million people worldwide, with about 1 million deaths annually. The magnitude of this public health problem is the principal reason for the great interest in immunity to parasites and for the development of immunoparasitology as a distinct branch of immunology.

Most parasites go through a complex life cycle, part of which is in humans (or other vertebrates) and part is in intermediate hosts such as flies, ticks, and snails. Humans are infected usually by bites from infected intermediate hosts or by sharing a particular habitat with an intermediate host. For instance, malaria and trypanosomiasis are transmitted by insect bites and schistosomiasis is transmitted by exposure to water in which infected snails reside.

A fundamental feature of most parasitic infections is their chronicity. There are many reasons for this, including weak natural immunity and the ability of parasites to evade or resist elimination by specific immune responses. Furthermore, many anti-parasite antibiotics are toxic and/or relatively ineffective. Individuals living in endemic areas require repeated chemotherapy because of continued exposure, and this is often not possible because of expense and logistical problems. Because of these reasons, attempts to develop prophylactic vaccines for parasites have long been considered an area of great priority for developing countries. The persistence of parasites in human hosts also leads to immunologic reactions that are chronic and may result in pathologic tissue injury as well as abnormalities in immune regulation. Therefore, some of the clinicopathologic consequences of parasitic infestations are due to the host response and not the infection itself.

Natural Immunity to Parasites

Protozoan and helminthic parasites that enter the blood stream or tissues are often able to survive and replicate because they are well adapted to resisting natural host defenses. The invertebrate stages of many parasites, which are recovered from the nonhuman intermediate hosts, activate the alternative pathway of complement and are lysed by the MAC. However, parasites recovered from the vertebrate, e.g., human, host are usually resistant to lysis by complement. This may be due to many reasons, including loss of surface molecules that bind complement or the acquisition of host regulatory proteins such as decay accelerating factor (DAF). Macrophages can phagocytose protozoa, but many pathogenic organisms are resistant to phagocytic killing and may even replicate within macrophages. The tegument of helminthic parasites makes them resistant to the cytocidal mechanisms of both neutrophils and macrophages.

Specific Immune Responses to Parasites

Different protozoa and helminths vary greatly in their structural and biochemical properties. It is, therefore, not surprising that different parasites elicit quite distinct specific immune responses, which are also different from the responses to bacteria and viruses. Although virtually every type of response to parasites has been reported, the major patterns of specific immunity to protozoa and helminths are the following:

1. *Production of specific IgE antibody and eosinophilia are frequently observed in helminthic infections.* Helminths such as *Nippostrongylus*, filaria, and schistosomes induce higher levels of IgE than any other infectious organisms. These responses are attributed to the propensity of helminths to stimulate specific $CD4^+$ helper T cells that secrete IL-4 and IL-5. Such helper T cells resemble the Th2 clones identified in mice that were mentioned in Chapter 9. The chronic antigenic stimulation caused by parasites may be especially effective at inducing the differentiation of T cells to functionally distinct subsets such as Th1 and Th2 cells. In mice infected with *Nippostrongylus brasiliensis* or *Schistosoma mansoni*, the elevation in serum IgE is blocked by injection of a neutralizing antibody specific for IL-4 and eosinophilia is inhibited by anti-IL-5 antibody. *In vitro* experiments suggest that IgE antibody-dependent cytotoxicity mediated by eosinophils may be particularly effective at resisting helminthic infections, because the major basic protein of eosinophil granules may be more toxic for helminths than the proteolytic enzymes and reactive oxygen species produced by neutrophils and macrophages. Thus, IgE antibody binds to helminths, eosinophils attach to these opsonized organisms by Fc receptors specific for IgE, the eosinophils are activated and secrete their granule contents, and the major basic protein lyses the parasites. Other antibodies and effector cells may also participate in host defense. Activated macrophages directly kill schistosome larvae through the action of nitrogen oxides and TNF. In rats, immunity to schistosomiasis can be passively transferred with IgG2 antibodies from infected animals. Such antibodies may activate the complement system or may opsonize parasites for phagocytosis or killing by activated macrophages and neutrophils.

2. *Some parasites and their products induce granulomatous responses with concomitant fibrosis. S. mansoni* eggs deposited in the liver stimulate $CD4^+$ T cells, which in turn activate macrophages as in DTH reactions. This results in the formation of granulomas around the eggs. The granulomas serve to contain the

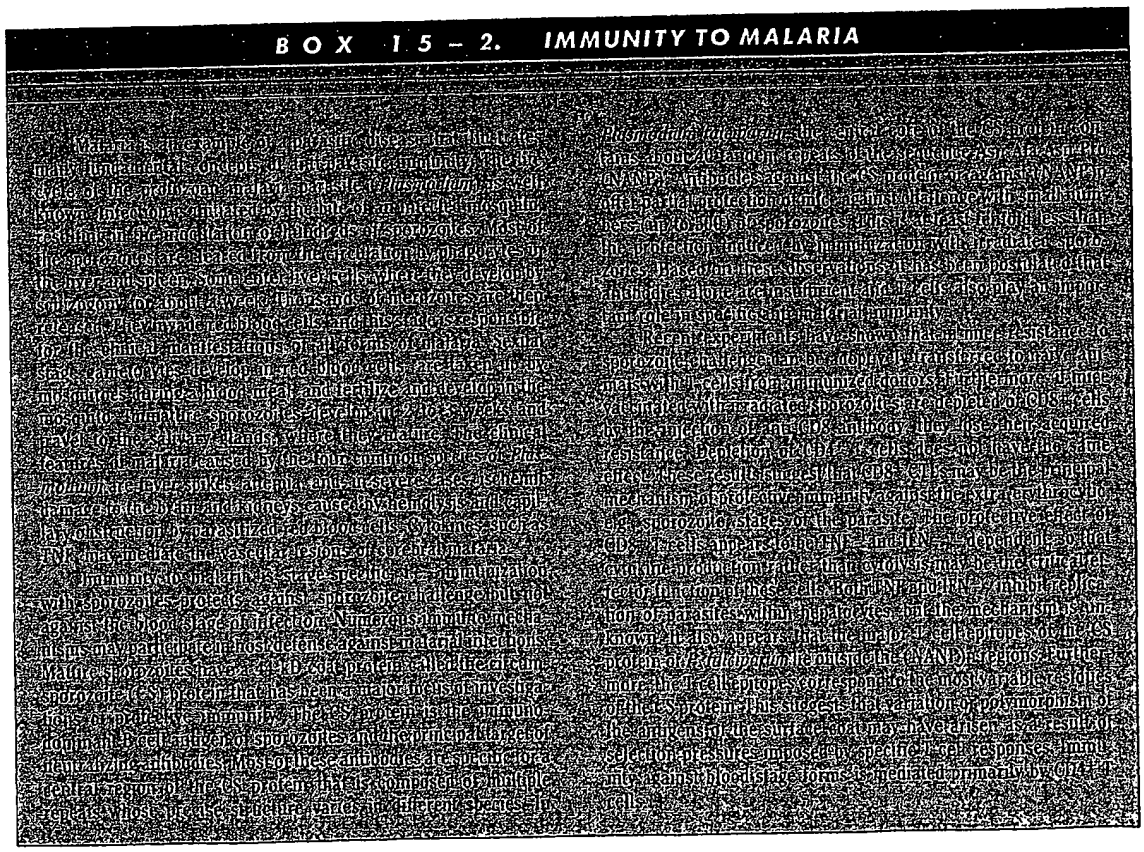
schistosome eggs, but severe fibrosis associated with this chronic cell-mediated immune response leads to disruption of venous blood flow in the liver, portal hypertension, and cirrhosis. In lymphatic filariasis, the parasites lodge in lymphatic vessels, leading to chronic cell-mediated immune reactions without granulomatous inflammation and ultimately to fibrosis. This results in lymphatic obstruction and severe, chronic lymphedema.

3. *$CD4^+$ helper T cells and cytokines may be involved in the resolution and exacerbation of some parasitic infections.* Perhaps the best documented example of this is *Leishmania major* infection in mice. Resistance to the infection is associated with the production of IFN-γ and TNF by $CD4^+$ T cells, whereas exacerbation of lesions is associated with high levels of IL-4. Inbred strains of mice that are susceptible to fatal leishmaniasis produce more IL-4 in response to the infection than resistant strains, and the injection of anti-IL-4 antibody induces resistance in the susceptible strains. In contrast, resistant strains produce higher amounts of IFN-γ and TNF and antibodies against these cytokines make the mice susceptible to the infection. IFN-γ and TNF presumably activate macrophages and enhance intracellular killing of leishmania. High levels of IL-4 may inhibit activation of macrophages by TNF and IFN-γ, and may block the production of cytokines, such as TNF, by the macrophages. In other parasitic infections, TNF may actually contribute to the pathology and clinical symptoms. For instance, fatal cerebral malaria in mice can be prevented by anti-TNF antibody and TNF production is associated with the cachexia seen in African trypanosomiasis. Such observations indicate that different parasites may stimulate unique patterns of specific T cell activation and cytokine production. Attempts to alter the outcome of these infections in experimental animals with cytokines or cytokine antagonists are going on in many laboratories at present.

4. *Protozoa that replicate inside cells may stimulate specific CTLs.* The CTL response to malaria is an important defense against the spread of this intracellular protozoon (Box 15-2). The poor efficacy of vaccination with malarial antigens is attributed to the inability of such immunizations to stimulate CTLs.

Chronic and persistent parasitic infestations are often associated with the formation of complexes of parasite antigens and specific antibodies. The complexes can deposit in blood vessels and kidney glomeruli, producing vasculitis and nephritis, respectively (see Chapter 18). Immune complex disease has been described in schistosomiasis and malaria. Malaria infections and African trypanosomiasis are also

BOX 15-2. IMMUNITY TO MALARIA



associated with the production of autoantibodies reactive with many self tissues. The myocarditis and neuropathy seen in Chagas' disease, which is caused by *Trypanosoma cruzi*, are probably autoimmune reactions because few or no parasites are present even in active lesions. Autoantibody production may also be secondary to polyclonal lymphocyte stimulation by the parasites (see Chapter 18). In contrast, in many cases of malaria and African trypanosomiasis, there is a severe and generalized suppression of the immune system. This may be secondary to the production of immunosuppressive cytokines by activated macrophages and/or T cells.

Evasion of Immune Mechanisms by Parasites

The ability of parasites to survive in vertebrate hosts reflects evolutionary adaptations that permit these organisms to evade or resist immune effector mechanisms. Different parasites have developed remarkably effective ways of resisting specific immunity. The most important of these fall into two categories: (1) parasites can reduce or alter their own antigenicity, or (2) they can actively inhibit host immune responses.

1. *Anatomic sequestration is commonly observed with protozoa.* Some (e.g., malaria parasites, *Toxoplasma*) survive and replicate inside cells, and others (like *Entamoeba* and *Trichinella*) develop cysts that are resistant to immune effectors. Some helminthic parasites reside in intestinal lumens and are sheltered from cell-mediated immune effector mechanisms. It is likely, however, that anatomic concealment is a temporary and only partially effective mechanism for evading immune responses.

2. *Antigen masking is an intriguing phenomenon in which a parasite, during its residence within a host, acquires on its surface a coat of host proteins.* The larvae of *S. mansoni* enter the skin and travel to the lungs and then into the circulation. By the time they enter the lungs, these larvae are coated with ABO blood group glycolipids and MHC molecules derived from the host. It is likely that many other host molecules attach to the surface of the schistosome larvae. It has been postulated that as a result of this coat of self proteins, parasite antigens are masked and the organism is seen as self by the host immune system. Although this is an interesting hypothesis, the significance of antigen masking is not clear because schistosome larvae do elicit specific immunity in vertebrate hosts.

3. *Parasites become resistant to immune effector mechanisms during their residence in vertebrate hosts.* Lung stage schistosome larvae develop a tegument that is resistant to damage by antibodies and complement or by CTLs directed against surface-bound antigens. This resistance is presumably due to a biochemical change in the surface coat. The structural complexity of the larval tegument has made it difficult to define the molecular alterations that are associated with acquired resistance. Infective forms of *T. cruzi* synthesize membrane glycoproteins similar to decay accelerating factor that inhibit complement activation. *Leishmania major* promastigotes induce rapid breakdown or release of the membrane attack complex thus reducing complement-mediated lysis. Parasites also evade macrophage killing by various mechanisms. *Toxoplasma gondii* inhibits phagolysosome fusion, and *T. cruzi* lyses the membranes of phagosomes and enters the cytoplasm before fusion with lysosomes can occur. Finally, some parasites express ectoenzymes that cleave bound antibody molecules and thus become resistant to antibody-dependent effector mechanisms.

4. *Parasites have developed effective mechanisms for varying their surface antigens during their life cycle in vertebrate hosts.* Two forms of antigenic variation are well defined.

a. The first is a stage-specific change in antigen expression, such that the mature tissue stages of parasites produce different antigens from the infective stages. For example, the infective sporozoite stage of malaria parasites is antigenically distinct from the merozoites that reside in the host and are responsible for chronic infection. By the time the immune system has responded to the infection, the parasite expresses new antigens and is no longer a target for immune elimination.

b. The most remarkable antigenic variation in parasites is the continuous variation of major surface antigens seen in African trypanosomes such as *Trypanosoma brucei* and *Trypanosoma rhodesiense.* Infected individuals show waves of blood parasitemia, and each wave consists of one antigenically unique parasite. The same phenomenon can be reproduced in experimental animals infected with a single clone of a trypanosome (Fig. 15-3). Thus, by the time the host produces antibodies against the parasite, an antigenically different organism has replicated. Over a hundred such recrudescent waves of parasitemia can occur in an infection. The major surface antigen of African trypanosomes is a glycoprotein dimer of approximately 50 kD, called the **variable surface glycoprotein** (VSG), which is attached to the surface by a phosphatidyl-inositol linkage. Trypanosomes contain more than 1000 different VSG genes, which vary markedly in their sequences except for the most C-terminal 50 amino acids (which are responsible for the surface linkage). Any one VSG gene is expressed in a particular clone at a particular stage of infection. Expression of a new gene may involve duplication and transposition of that gene to a more telomeric chromosomal site at which active transcription ensues (Fig. 15-1). This mechanism is different from the gene conversion events that lead to variation of antigens in the pili of gonococci. However, gene conversion and activation of previously silent VSG genes are additional mechanisms that may contribute to antigenic variation. Continuous antigenic variation in trypanosomes is neither induced by nor dependent on the specific antibody response and is probably due to a programmed variation in the expression of VSG genes. The molecular

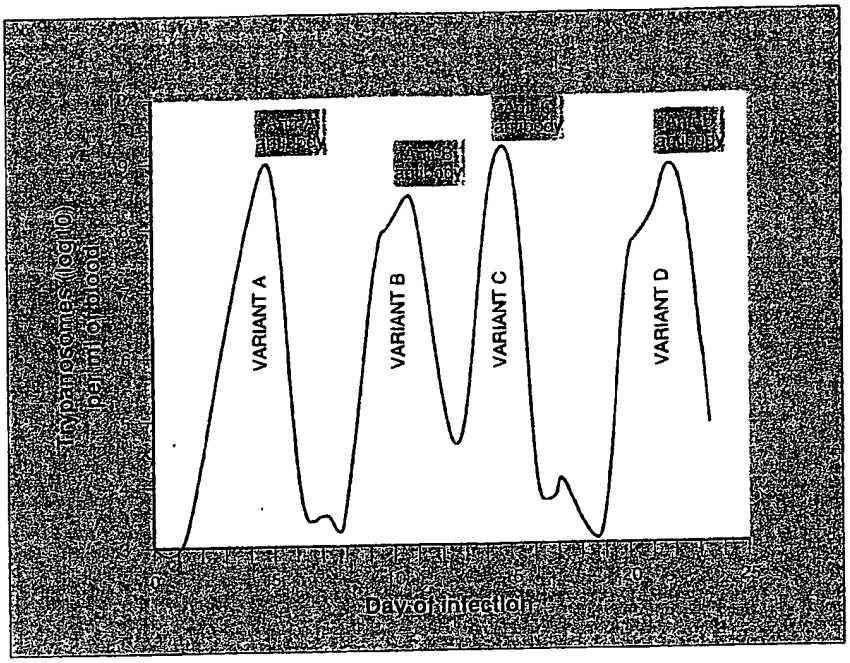


FIGURE 15-3. Parasitemia following trypanosome infections. In a mouse infected experimentally with a single clone of Trypanosoma rhodesiense, *the blood parasite counts show cyclical waves. Each wave is due to a new antigenic variant of the parasite (labeled A, B, C, and D) that expresses a new VSG, and each decline is a result of a specific antibody response to the variant. The durations of peak antibody production as shown are approximate. Similar waves of parasitemia are seen in natural infections in humans. (Courtesy of Dr. John Mansfield, University of Wisconsin, Madison.)*

mechanisms that regulate this phenomenon are the focus of active investigation in many laboratories.

One consequence of antigenic variation in parasites is that it is difficult to effectively vaccinate individuals against these infections. In fact, prophylactic immunization against one parasite antigen may itself stimulate additional antigenic variation, as has been seen in experimental malarial infections.

5. *Parasites shed their antigenic coats, either spontaneously or after the binding of specific antibodies.* Examples of active membrane turnover and loss of surface antigens have been described with *Entamoeba histolytica*, schistosome larvae, and trypanosomes. Shedding of antigens and bound antibodies renders the parasites relatively resistant to immune effector mechanisms.

6. *Parasites alter host immune responses by multiple mechanisms.* Specific anergy to parasite antigens has been described in severe schistosomiasis involving the liver and spleen and in filarial infections. The mechanisms of immunologic unresponsiveness in these patients are not well understood. In lymphatic filariasis, infection of lymph nodes with subsequent architectural disruption may contribute to deficient immunity. More nonspecific and generalized immunosuppression, e.g., in systemic leishmaniasis, has been mentioned earlier. It has been variously attributed to abnormalities in cytokine production, deficient T cell activation, immunosuppressive macrophages, and "suppressor cells." Better structural definition of parasite antigens and analysis of specific lymphocyte responses are now being done by many research groups.

The worldwide implications of parasitic infestations for health and economic development are well appreciated. Attempts to develop effective vaccines against these infections have been actively pursued for many years (Box 15-3). Although the progress has been slower than one would have hoped, elucidation of the fundamental mechanisms of immune responses to and immune evasion by parasites holds great promise for the future.

SUMMARY

The interaction of the immune system with infectious organisms is a dynamic interplay of host mechanisms aimed at eliminating infections and microbial strategies designed to permit survival in the face of powerful effector mechanisms. Different types of infectious agents stimulate distinct patterns of immune responses and have evolved unique mechanisms for evading specific immunity.

The principal protective immune response against extracellular bacteria consists of specific antibodies, which opsonize the bacteria for phagocytosis and activate the complement system. Toxins produced by such bacteria are also neutralized and eliminated by specific antibodies. Some bacterial toxins are powerful inducers of cytokine production, and cytokines account for much of the systemic pathology associated with severe, disseminated infections with these microbes.

Intracellular bacteria are capable of surviving and replicating within host cells, including phago-

BOX 15–3. STRATEGIES FOR VACCINE DEVELOPMENT

cytes, because they have developed mechanisms for resisting lysosomal degradation. Immunity against these microbes is principally cell-mediated and consists of $CD4^+$ T cells activating macrophages (as in delayed type hypersensitivity) as well as $CD8^+$ cytolytic T lymphocytes. The characteristic pathologic response to infection by intracellular bacteria is granulomatous inflammation.

Viruses are obligatory intracellular microbes. Natural immunity against viruses is mediated by type I interferons and NK cells. Specific antibodies protect against viruses early in the course of infection. However, the major defense mechanism against established infections consists of specific CTLs. CTLs effectively lyse infected cells and may contribute to tissue injury even when the infectious virus is not cytopathic by itself.

Animal parasites, such as protozoa and helminths, give rise to chronic and persistent infections, because natural immunity against them is weak and because parasites have evolved multiple mechanisms for evading and resisting specific immunity. The structural and antigenic diversity of pathogenic parasites is reflected in the heterogeneity of the specific immune responses they elicit. Different parasites induce specific IgE antibodies and eosinophilia, granulomatous inflammation, cytokine production, and specific CTLs. Parasites evade the immune system by masking and shedding their surface antigens and by varying their antigens during residence in vertebrate hosts. In addition, various parasites cause specific and generalized suppression of lymphocyte activation. The chronicity of parasitic infestations often leads to secondary immunopathologic consequences, including the formation of immune complexes and the development of autoimmunity.

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Exhibit 2-2

CHAPTER SIXTEEN

IMMUNITY TO TISSUE TRANSPLANTS

Transplantation is the process of taking cells, tissues, or organs, called a **graft,** from one individual and placing them into a (usually) different individual. The individual who provides the graft is referred to as the **donor,** and the individual who receives the graft is referred to as either the **recipient** or the **host.** If the graft is placed into its normal anatomic location, the procedure is called *orthotopic transplantation;* if the graft is placed in a different site, the procedure is called *heterotopic transplantation.* Transfusion is transplantation of circulating blood cells and/or plasma from one individual to another.

Although attempts at transplantation date back to ancient times, the impetus behind modern transplantation was World War II and the Battle of Britain. Royal Air Force pilots were often severely burned when their planes crashed. The mortality associated with burns corresponds to the size of the area of skin that has been injured, and survival can be improved if burned skin is replaced. For this reason, British doctors turned to skin transplantation from other human donors as a mode of therapy. However, attempts to replace damaged skin with skin from unrelated donors were uniformly unsuccessful. Over a matter of several days, the transplanted skin would undergo necrosis and fall off. This problem led many investigators, including Peter Medawar, to study skin transplantation in animal models. These experiments established that the failure of skin grafting was caused by an inflammatory reaction that was called **rejection.** More importantly, several features indicated that *rejection is a form of specific immunity.* The key experimental results may be summarized as follows (Table 16-1):

1. A skin graft transplanted between genetically unrelated individuals, e.g., from a strain A mouse to a strain B mouse, is rejected by a naive host in 7 to 10 days. This process is called **first set rejection.** A subsequent skin graft transplanted from the same donor to the same recipient is rejected more rapidly, i.e., in only 2 or 3 days. This accelerated response, called **second set rejection,** is an example of memory, one of the cardinal features of acquired immunity.

2. Second set rejection ensues if the first and second skin grafts are derived from the same donor or from genetically identical donors, e.g., strain A mice. However, if the second graft is derived from an individual unrelated to the donor of the first graft, e.g., strain C, there is no second set rejection; the new graft elicits only a first set rejection. Thus, the phenomenon of second set rejection shows specificity, another cardinal feature of acquired immunity.

3. The ability to mount second set rejection against a graft from strain A mice can be adoptively transferred to a naive strain B recipient by immunocompetent lymphocytes taken from a strain B animal previously exposed to a graft from strain A mice. This experiment demonstrated that second set rejection is mediated by sensitized lymphocytes and provided the definitive evidence that rejection is a form of acquired immunity.

Transplant immunologists have developed a vocabulary to describe the kinds of cells and tissues encountered in the transplant setting. A graft transplanted from one individual to the same individual is called either an **autologous graft** (shortened to **autograft**) or an **isograft.** A graft transplanted between two genetically identical or syngeneic individuals is called a **syngeneic graft** (or **syngraft.**) A graft transplanted between two genetically different individuals of the same species is called an **allogeneic graft** (or **allograft**). A graft transplanted between individuals of different species is called a **xenogeneic graft** (or **xenograft**). The molecules that are recognized as foreign on allografts are called **alloantigens,** and those on xenografts are called **xenoantigens.** The lymphocytes or antibodies that react with alloantigens or xenoantigens are described as being **alloreactive** or **xenoreactive,** respectively.

The remainder of this chapter focuses on allogeneic transplantation because it is far more commonly practiced and better understood than xenogeneic transplantation. We will consider both the basic immunology and some aspects of the clinical practice of transplantation. Transplantation of organs such as kidney, heart, and liver is currently in widespread use, and the practice is growing. In addition, the transplantation of many other organs is now being attempted. The immunology of transplantation is important for two reasons. First, the immunologic rejection response, along with problems of organ procurement, are the two major barriers to transplantation today. Second, although the encounter with alloantigens appears to be an unlikely happenstance in the

TABLE 16-1. First Set and Second Set Allograft Rejection

Animal	Skin Graft Donor	Recipient		Rejection
		Strain	*Prior Treatment*	
1	Strain A	Strain B	None	Slow (first set)
2	Strain A	Strain B	Sensitized by previous graft from strain A donor	Rapid (second set); demonstration of immunologic memory
3	Strain A	Strain B	Injected with lymphocytes from animal No. 1	Rapid (second set); demonstration of role of lymphocytes in graft rejection
4	Strain C	Strain B	Sensitized by previous graft from strain A donor	Slow (first set); demonstration of immunologic specificity

normal life of an organism, the immune response to allogeneic molecules is very strong and has therefore been a useful model for elucidating the mechanisms of lymphocyte activation.

TRANSPLANTATION IMMUNOLOGY

The immune response to alloantigens can be either cell-mediated or humoral. In general, cell-mediated immune reactions are more important for rejection of transplanted organs, but antibodies may contribute. Most studies of the immune responses to tissue transplants have focused on T cell responses to allogeneic molecules. There are three major questions addressed by these studies:

1. What antigens in grafts stimulate alloreactivity?
2. What types of lymphocytes respond to foreign transplants?
3. Why do individuals react against tissues that they do not encounter normally?

Molecular Basis of Allogeneic Recognition

In Chapter 5, we presented evidence that recognition of transplanted cells as self or foreign is determined by inheritance of co-dominant genes. This conclusion was based on the results of experimental transplantation between inbred strains of mice.

1. Cells or organs transplanted between individuals of the same inbred strain of mice are never rejected.
2. Cells or organs transplanted between individuals of different inbred strains of mice are almost always rejected.
3. The offspring of a mating between two different inbred strains will never reject grafts from either parent. In other words, an (A × B)F1 animal will not reject grafts from an A or B strain animal.
4. A graft derived from the offspring of a mating between two different inbred strains will almost always be rejected by either parent. In other words, a graft from an (A × B)F1 animal will be rejected by either an A or a B strain animal.

These genetic experiments led to the hypothesis that certain polymorphic gene products, co-dominantly expressed on a graft, are recognized by the immune system to identify a graft as self or foreign. Co-dominant expression means that an (A × B)F1 animal expresses both A strain and B strain alleles. This is why an (A × B)F1 animal is tolerant to both A and B strain grafts and why both A and B strain animals will recognize an (A × B)F1 graft as foreign. As described in Chapter 5, George Snell and colleagues were able to identify about 40 polymorphic genes that served as the molecular targets of rejection in mice. Specifically, they found that polymorphic molecules encoded by genes in histocompatibility locus 2 (H-2), now known as the mouse major histocompatibility complex, were responsible for almost all the strong (rapid) rejection reactions. *As many as 2 per cent of a host's T cells are capable of recognizing and responding to a single foreign MHC molecule.* This high frequency of T cells reactive with allogeneic MHC molecules is the reason why allograft rejection is a strong response *in vivo*.

An important question in transplantation is the molecular structure of the determinants on allogeneic MHC molecules that are recognized by alloreactive T cells. We now know that mature T cells express receptors that are specific for foreign peptides bound to the peptide-binding cleft of self class I or class II MHC molecules. Any given T cell receptor (TCR) simultaneously recognizes amino acid residues of the bound foreign peptide and residues of self MHC molecules. Foreign MHC molecules differ from self MHC molecules by variations (polymorphisms) in amino acid sequence, and, as we noted in Chapter 5, these polymorphic residues are confined to the top, sides, and floor of the peptide-binding cleft. The determinants of foreign MHC molecules recognized by specific alloreactive T cells are formed largely by these polymorphic residues. It is believed that the three-dimensional surface of the determinant recognized on the foreign MHC molecules resembles self MHC plus a bound foreign peptide. Thus, *recognition of foreign MHC molecules is a cross-reaction of a normal T cell receptor which was selected to recognize self MHC plus foreign peptide.* Three kinds of experiments support this conclusion:

1. A T cell clone or hybridoma that contains one set of functionally rearranged T cell receptor genes specific for self MHC plus a foreign peptide may also recognize one or more foreign MHC molecules in the absence of the specific foreign peptide.
2. Monoclonal antibodies reactive with idiotypic determinants on the TCR molecule of such a T cell clone or hybridoma may inhibit recognition of both self MHC-associated foreign peptide and foreign MHC molecules.
3. Transfection of rearranged α and β T cell receptor genes into a recipient T cell confers specificity both for self MHC plus foreign peptide and for foreign MHC molecules.

Because in these *in vitro* systems exogenously added foreign peptides were not necessary for allorecognition, the results initially seemed to support the conclusion that bound peptide does not contribute to the determinant formed by a foreign MHC molecule. However, MHC molecules expressed on cell surfaces may always contain bound peptides. Even in artificial systems, such as lipid bilayers containing purified foreign MHC molecules, peptides could be provided by the culture medium or by the responding T cell or could remain associated with the MHC molecules through purification. Moreover, some alloreactive T cell clones have now been shown to be specific for foreign MHC plus bound peptide. The peptides recognized in association with foreign MHC molecules may be self peptides because thymic education

does not produce tolerance to self proteins plus foreign MHC. The importance of the contribution of bound peptide to recognition by most alloreactive T cells is not yet known.

It is surprising that many more mature T cells recognize particular foreign MHC molecules than recognize any specific foreign peptide. This raises the question of why alloreactive cells are so common. Two explanations have been offered to account for widespread cross-reactivity.

1. *Foreign MHC molecules differ from self MHC molecules at multiple different amino acid residues, each of which individually or in combination may produce a determinant recognized by a different cross-reactive T cell clone.* Thus, each foreign MHC molecule is recognized by multiple clones of T cells whose receptors are specific for different foreign peptides in association with self MHC molecules. In this model, widespread cross-reactivity is caused entirely by multiple variations in the sequence of the foreign MHC molecule. For example, foreign MHC molecule X will be recognized by T cell 1, specific for self MHC molecule Y plus peptide A and by T cell 2, specific for self MHC molecule Y plus peptide B.

2. *Different bound self peptides, in combination with one foreign MHC molecule, may produce determinants recognized by different cross-reactive T cell clones.* The result, once again, is that many T cell clones recognize and respond to each foreign MHC molecule, but in this model, widespread cross-reactivity is caused by many different self peptides combining with a lesser degree of variation in the sequence of the foreign MHC molecule. For example, foreign MHC molecule X plus self peptide A will be recognized by T cell 1, specific for self MHC molecule Y plus foreign peptide B and foreign MHC molecule X plus self peptide C will be recognized by T cell 2 specific for self MHC molecule Y plus foreign peptide D.

These two models of alloantigen recognition are not mutually exclusive. It seems likely that associated self peptides will be more important (model 2) in those cases in which the self and foreign MHC molecules are structurally similar, and less important (model 1) when the self and foreign MHC molecules are structurally dissimilar to each other.

Polymorphic alloantigens other than MHC molecules generally produce weak or slower (gradual) rejection reactions and are called minor histocompatibility antigens. Most minor histocompatibility antigens are proteins that are processed and presented to host T cells in association with either self MHC or graft MHC molecules. In contrast, foreign MHC molecules can be recognized directly by host T cells without any requirement for processing or association with self MHC molecules.

Cellular Basis of Allogeneic Recognition

Vigorous rejection reactions of allografts generally result from recognition of the transplanted tissues by both $CD4^+$ and $CD8^+$ T cells. The **mixed leukocyte reaction** (MLR) has been a useful model for understanding the cellular basis of alloantigen recognition by different T cell subpopulations.

The Mixed Leukocyte Reaction

As we have discussed above, MHC genes were initially identified for their role in graft rejection, which is often a T cell–mediated process. The MLR is an *in vitro* model of T cell recognition of foreign MHC gene products and is used as a predictive test of cell-mediated graft rejection.

The MLR is induced by culturing mononuclear leukocytes (which include T cells, B cells, mononuclear phagocytes, and dendritic cells), from one individual or inbred strain with mononuclear leukocytes derived from another individual or strain. In humans, these cells are typically isolated from peripheral blood; in the mouse or rat, mononuclear leukocytes are usually purified from spleen or lymph nodes. If there are differences in the alleles of the MHC genes between the two individuals, a large proportion of the mononuclear cells will proliferate over a period of 4 to 7 days. This proliferative response, usually measured by incorporation of ^{3}H-thymidine into DNA during cell replication, is called the **allogeneic MLR** (Fig. 16–1). In the experiment described above, the cells from each donor react and proliferate against the other, resulting in a "two-way MLR." To simplify the analysis, one of the two mononuclear leukocyte populations can be rendered incapable of proliferation, either by gamma irradiation or by treatment with mitomycin C, an antimitotic drug, prior to culture. In this "one-way MLR," the treated cells serve exclusively as **stimulators** and the untreated cells, still capable of proliferation, serve as the **responders.**

Two populations of alloreactive T cells are stimulated during an allogeneic MLR, and each responding T cell subset recognizes a different MHC gene product. One type of T cell expresses the CD8 but not the CD4 molecule, usually functions as a cytolytic T lymphocyte (CTL), and is indistinguishable from self class I MHC–restricted CTLs specific for foreign protein antigens. The CTLs generated during an allogeneic MLR lyse target cells derived from the same individual or strain as the original stimulator cell population. The molecules on stimulator and target cells that are recognized by $CD8^+$ CTL are the class I MHC molecules, namely, HLA–A, B, or C in humans or H-2K, D, or L in mice. Several lines of evidence have indicated that foreign class I MHC gene products are the actual molecular targets recognized by the $CD8^+$ CTLs generated in the MLR:

1. If there are no differences in class I MHC alleles between the stimulator and responder cell populations in the MLR, $CD8^+$ CTLs are not generated.
2. The CTLs generated against one stimulator cell population will lyse third-party target cells only if these targets share a class I MHC allele with the original stimulators.
3. Antibodies directed against class I MHC allelic

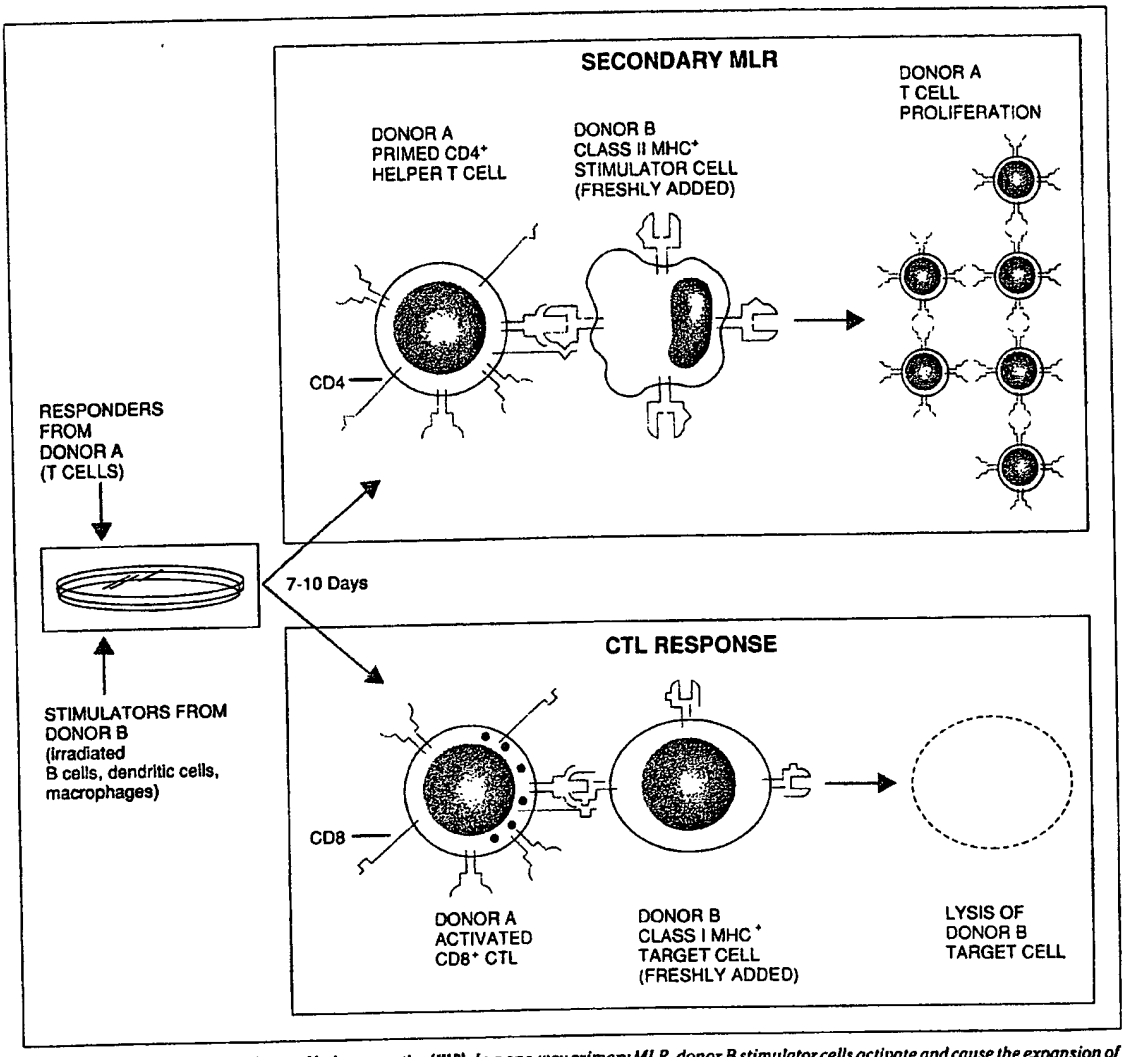

FIGURE 16–1. Responder T cells in the mixed leukocyte reaction (MLR). *In a one-way primary MLR, donor B stimulator cells activate and cause the expansion of two types of donor A responder T cells: CD4+ helper T cells, which can be detected in a secondary MLR by rapid proliferation to antigen-presenting cells (APCs) bearing donor B class II molecules; and CD8+ cytolytic T lymphocytes (CTLs), which can be detected in a specific killing assay using target cells bearing donor B class I molecules.*

gene products on the stimulator cells protect target cells against lysis.

4. Transfection and expression of an allelic class I MHC gene can render a cell susceptible to lysis by a CTL population specific for that allele.

The full differentiation of CTLs in the MLR requires stimulation by class I MHC molecules as well as help that is optimally provided by CD4+ T cells present in the same culture (see Chapter 12).

Within the CD8+ CTL population derived from an MLR, each individual CTL is specific for only one particular class I MHC gene product. However, the bulk population contains CTLs directed against all class I MHC allelic differences between the original stimulator and responder populations. Furthermore, in an outbred individual, all the class I MHC alleles inherited from both parents are co-dominantly expressed on every class I–expressing cell, so that an individual target cell can be lysed by several different CTLs, each with a different class I MHC specificity.

The second type of T cell that is generated during the MLR was initially called a primed responder cell, because when such T cells are recultured with stimulator cells from the same donor individual (or strain) used in the original MLR, a secondary MLR ensues that is stronger and more rapid; i.e., peak proliferation occurs by 2 or 3 days. It is now appreciated that

primed responder cells are interleukin-2 (IL-2)-producing T cells that play a role in the development of $CD8^+$ CTLs. Such T cells express the CD4 but not the CD8 surface molecule and are indistinguishable from IL-2-producing $CD4^+$ helper T cells specific for foreign protein antigens. Alloreactive $CD4^+$ helper T cells are specific for allogeneic class II MHC molecules, i.e., HLA-DR, DP, and DQ in humans and I-A and I-E in mice. The class II MHC molecules have been established as the molecules seen by the $CD4^+$ helper cells by the same kinds of evidence that established class I MHC molecules as the targets of $CD8^+$ CTL.

1. Alloreactive $CD4^+$ T cells are stimulated only if there are differences in class II MHC alleles between the original stimulator and responder cells in the primary MLR.
2. Alloreactive $CD4^+$ T cells respond to third-party stimulator cells only if they share class II MHC alleles with the original stimulator population.
3. Antibodies directed against class II MHC gene products prevent development of the secondary MLR.
4. Transfection of appropriate allelic class II MHC genes can convert a cell from a non-stimulator to a stimulator of a $CD4^+$ T cell population specific for that MHC allele.

Alloreactive $CD4^+$ T cells, like self MHC-restricted antigen-specific helper cells, can be stimulated only by cells that express class II MHC molecules and provide costimulatory signals. The most efficient stimulators are dendritic cells, B lymphocytes, and mononuclear phagocytes. In humans, vascular endothelial cells are also able to stimulate proliferative responses of alloreactive $CD4^+$ T cells. Each individual $CD4^+$ helper T cell is specific for one particular class II gene product. However, the bulk population contains helper cells reactive with all class II allelic differences between the original stimulator and helper cell population. Furthermore, since there is no allelic exclusion of class II genes on individual cells and all the parental alleles are co-dominantly expressed, one stimulator cell can activate several different helper T cells, each with a different class II MHC specificity.

It should be emphasized that the functional subdivision of $CD4^+$ and $CD8^+$ alloreactive T cells into helper cells and CTLs is not absolute. $CD4^+$ class II specific CTLs can be detected in the MLR, particularly in humans. Moreover, at least some $CD8^+$ T cells produce IL-2, γ-interferon (IFN-γ), tumor necrosis factor (TNF), and lymphotoxin (LT), which is similar to the cytokine profile of many $CD4^+$ cells. As discussed in Chapters 6 and 7, the same exceptions have been found for self MHC-restricted T cells specific for foreign protein antigens.

A more specific analysis of the role of class I and class II molecules in allogeneic immune responses has been performed by considering the one-way MLR when only isolated class I or class II differences exist between the stimulator and responder cell populations (Table 16-2). In the extreme case, this has been done by using cells from mouse strains that differ only by a small mutation in a single class I or class II gene product. Proliferation is strongest when stimulators and responders differ by a class II gene product and can directly stimulate $CD4^+$ T cells. Fewer CTLs arise in this instance, and many of these are $CD4^+$ class II-specific CTLs. When only class I differences exist between stimulator and responder cells, the proliferative response is small. Nevertheless, there is a proliferative response and $CD8^+$ CTLs specific for the class I difference do arise. In this situation, proliferation and the development of CTLs may be mediated by cytokines produced by the $CD8^+$ T cells themselves responding to the foreign class I molecules. Alternatively, if class II MHC^+ accessory cells are present, they may take up, process, and present foreign class I molecules in the form of peptides associated with self class II molecules. In this setting, the foreign class I molecules behave like other foreign protein antigens that are recognized by $CD4^+$ T cells present in the responder population. This process is far less efficient than direct recognition of foreign class II molecules. Therefore, stimulator cell populations that differ from

TABLE 16-2. Induction of Mixed Leukocyte Reaction by Class I and Class II Major Histocompatibility Complex (MHC) Mismatches

MHC Differences Between Stimulator and Responder Cells			Induction of	
Class I	*Class II*	Proliferation	*Primed Helper (Cytokine-Producing) Cells*	*CTLs*
Yes	Yes	++++	++++ ($CD4^+$, class II-specific)	++++ ($CD8^+$, class I-specific)
Yes	No	+	+ ($CD8^+$, class I-specific)	+++ ($CD8^+$, class I-specific)
No	Yes	+++	+++ ($CD4^+$, class II-specific)	+ ($CD4^+$, class II-specific)

In a one-way MLR between stimulator and responder cells that differ at class I or class II MHC loci or both, the major types of cytokine-producing helper (proliferating) cells and CTL are different.

Abbreviations: CTL, cytolytic T lymphocyte.

the responders at both the class I and class II MHC loci induce many more allospecific CTLs than stimulators that differ at only class I loci.

Two additional points about the MLR should be noted:

1. *The MLR is the only* in vitro, *antigen-specific response of T cells that can be readily observed without prior immunization* in vivo. This is because T cells reactive with allogeneic MHC molecules are much more numerous in an unstimulated population than are the T cells specific for any single foreign protein antigen. In addition, an allogeneic stimulator cell may express a large number of foreign MHC molecules that are capable of stimulating alloreactive T cells. In contrast, on an antigen presenting cell (APC) that is normally presenting a foreign peptide antigen, a minor fraction of the MHC molecules may be complexed with the specific peptide. As a result, many more alloreactive than foreign antigen-specific, self MHC-restricted T cells are stimulated by such APCs.

2. Although the MLR is initiated by allogeneic stimulation, the majority of helper T cells and CTLs that proliferate in the primary MLR are actually not specific for the allogeneic MHC gene products on the stimulator cells. Rather, they are driven to proliferate by growth factors, such as IL-2, produced by a small number of specifically stimulated alloreactive cells. However, the specifically stimulated alloreactive cells preferentially increase in number, compared with any other T cell, and thus become the only cells sufficiently numerous to be detectable as CTLs or primed helper cells after the primary MLR.

Stimulation of Alloreactive T Cells *in vivo*

In the case of allografts that differ from hosts at both class I and class II loci, both $CD8^+$ and $CD4^+$ T cells are activated by recognition of alloantigens of the grafts. $CD8^+$ cells recognize foreign class I MHC molecules, which are expressed by all the cells in the graft. The differentiation of these CTLs is largely dependent upon $CD4^+$ T cells being stimulated by allogeneic class II molecules present on APCs in the allograft. Therefore, one can predict that tissue allografts that stimulate strong rejection contain class II-bearing APCs.

The importance of APCs in stimulating an alloantigenic immune response *in vivo* has most clearly been demonstrated by experiments in rodents. If class II-bearing cells (which include APCs) are removed from a graft prior to transplantation, such grafts are usually rejected slowly or may even be accepted despite class I MHC differences. (Experimentally, class II bearing cells may be eliminated from such grafts by several treatments, including prolonged culture; treatment with anti-class II antibody plus complement; or, in some cases, extensive perfusion of graft blood vessels to "wash out" the APCs.) When rat kidney allografts are purged of class II-bearing cells by perfusion, infusion of dendritic cells derived from the organ donor concomitant with transplantation restores allorecognition and leads to rapid rejection. These observations have led to several conclusions, collectively described as the **passenger leukocyte hypothesis.**

BOX 16-1. IMMUNITY TO AN ALLOGENEIC FETUS

The mammalian fetus, except in those instances in which the mother and father are syngeneic, will express paternally-inherited antigens that are allogeneic to the mother. Nevertheless, fetuses are not normally rejected by the mother. An understanding of how the fetus escapes the maternal immune system may be relevant for transplantation.

Three experimental observations indicate that the anatomic location of the fetus is a critical factor in the absence of rejection:

1. Wholly allogeneic fetal blastocysts that lack any maternal genes can successfully develop in a pregnant or pseudopregnant mother. Thus, neither specific maternal nor paternal genes are necessary for survival of the fetus.
2. Hyperimmunization of the mother with cells bearing paternal antigens does not compromise placental and fetal growth.
3. Pregnant mothers are able to recognize and reject allografts syngeneic to the fetus placed at extrauterine sites without compromising fetal survival.

The failure to reject the fetus has focused attention upon the region of physical contact between the mother and fetus. The fetal tissues of the placenta that most intimately contact the mother may be classified as *vascular trophoblast*, which is exposed to maternal blood for purposes of mediating nutrient exchange, and *implantation site trophoblast*, which diffusely infiltrates the uterine lining (decidua) for purposes of anchoring the placenta to the mother.

One simple explanation for fetal survival is that trophoblast cells fail to express paternal MHC molecules. So far, class II molecules have not been detected on trophoblast. In mice, cells of the implantation trophoblast, but not vascular trophoblast, do express paternal class I molecules. In humans, the situation may be more complex, in that trophoblast cells may express only a non-polymorphic class I-like molecule. However, even if these cells do express classical MHC molecules, they may lack costimulator functions and fail to act as APCs.

A second explanation for lack of rejection is that the uterine decidua may be an immunologically privileged site that is not accessible to functional T cells. In support of this idea is the observation that mouse decidua is highly susceptible to infection by *Listeria monocytogenes* and cannot support a delayed type hypersensitivity response. The basis of immunologic privilege is clearly not a simple anatomic barrier because maternal blood is in extensive contact with trophoblast. Rather, the barrier is likely to be functional inhibition. Cultured decidual cells directly inhibit macrophage and T cell functions, perhaps by producing inhibitory cytokines such as transforming growth factor-β (see Chapter 11). Some of these inhibitory decidual cells may be resident suppressor T cells, although the evidence for this proposal is not convincing.

1. In order to stimulate a rejection reaction, host $CD4^+$ helper T cells are activated by foreign cells that express allogeneic class II molecules and provide costimulators. The $CD4^+$ T cells then stimulate the growth and differentiation of alloreactive $CD8^+$ CTLs.
2. The cells that provide such costimulatory functions are traditional APCs and are usually present as "passenger leukocytes" carried along with the graft.
3. Elimination of passenger leukocytes serves to reduce the incidence and severity of rejection, by reducing the activation of helper T cells.

Although the role of passenger leukocytes is well documented in rodents, attempts to remove such cells have not been useful in human transplantation. The probable explanation is that human, but not rodent, endothelial cells provide costimulator functions, activate alloreactive $CD4^+$ helper T cells, and are sufficient to initiate rejection even in the absence of passenger leukocytes.

In contrast to T cell alloreactivity, much less is known about the mechanisms that lead to the production of alloantibodies against foreign MHC molecules. Presumably, B cells specific for alloantigens are stimulated by mechanisms similar to those involved in stimulation of B cells reactive with other foreign proteins.

Before we conclude this section of the chapter, we should point out that many of the issues that arise in discussing alloreactivity and graft rejection are also relevant to maternal-fetal interactions. The fetus expresses paternal MHC molecules and is, therefore, semiallogeneic to the mother. Nevertheless, the fetus is not rejected by the maternal immune system. Many possible mechanisms have been proposed to account for this, and it is not yet clear which of these mechanisms are the most significant (Box 16-1, p. 323).

Effector Mechanisms in Allograft Rejection

So far we have described the molecular basis of allogeneic recognition and the cells involved in the recognition of and responses to allografts. We now turn to a consideration of the effector mechanisms used by the immune system to reject allografts. In different experimental models, alloreactive $CD4^+$ or $CD8^+$ T cells or specific alloantibodies are all capable of mediating allograft rejection upon adoptive transfer. Furthermore, graft rejection can be inhibited by anti-CD4 or anti-CD8 antibodies. It is now clear that these different immune effectors cause graft rejection by different mechanisms.

1. Alloreactive $CD4^+$ T cells can recruit and activate macrophages, initiating graft injury by a delayed type hypersensitivity (DTH) response (see Chapter 12).
2. Alloreactive $CD8^+$ T cells directly lyse graft endothelial and parenchymal cells.
3. Alloantibodies activate the complement system and injure graft blood vessels.

For historical reasons, graft rejection is usually classified on the basis of histopathology rather than on immune effector mechanisms. Based upon the experience of renal transplantation, the histopathologic pattern is called hyperacute, acute, or chronic.

The names of the various forms of rejection imply a temporal sequence of events, but this is not strictly true. Although hyperacute rejection is always a very rapid process immediately following transplantation, acute and chronic rejection can occur at almost any time after transplantation. Indeed, acute and chronic rejection often co-exist in the same graft. Histology, rather than the length of time following transplantation, is the major criterion for classifying rejection reactions. However, in the current era of renal transplantation, in many centers biopsies are performed less frequently than in the past and diagnosis is often made on the basis of clinical features and post-transplantation time without histologic confirmation.

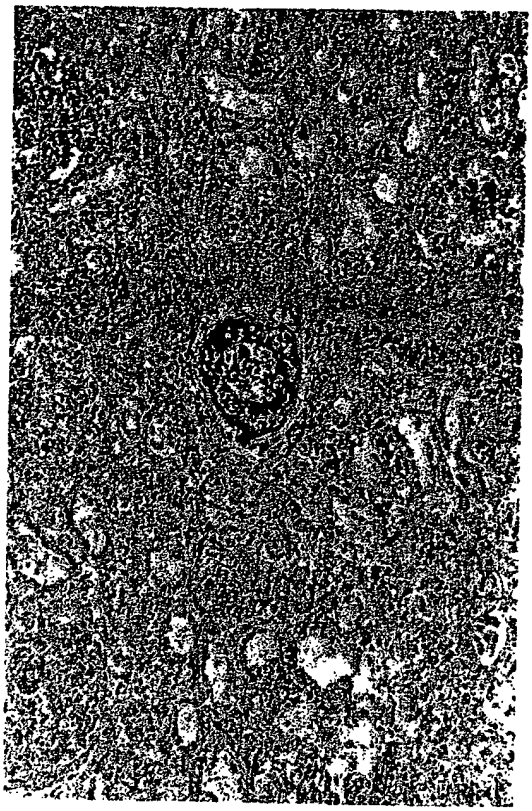

FIGURE 16-2. Hyperacute rejection in the kidney. Preformed antibodies reactive with vascular endothelium of a kidney allograft activate complement and trigger rapid intravascular thrombosis and necrosis of the vessel wall, often preceding the development of an inflammatory reaction. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston.)

HYPERACUTE REJECTION

Hyperacute rejection is characterized by rapid thrombotic occlusion of the graft vasculature that begins within minutes after host blood vessels are anastomosed to graft vessels (Fig. 16-2, p. 324). Thrombosis occurs prior to the development of inflammation. *Hyperacute rejection is mediated by pre-existing antibodies that bind to endothelium and activate complement.* The endothelial cells are stimulated to secrete high molecular weight forms of von Willebrand factor that mediate platelet adhesion and aggregation. Complement activation also leads to endothelial cell injury, initiation of coagulation, and exposure of subendothelial basement membrane proteins that activate platelets. These processes contribute to thrombosis and vascular occlusion, and the organ suffers irreversible ischemic damage.

In the early days of transplantation, hyperacute rejection was often mediated by pre-existing IgM antibodies which were present at high titer prior to any exposure to alloantigens. Such "natural antibodies" are believed to arise in response to carbohydrate antigens expressed by the bacteria that normally colonize the bowel. The best known examples of such antibodies are those directed against the ABO blood group antigens expressed on red blood cells (Box 16-2). ABO antigens are also expressed on vascular endothelial cells. Today, hyperacute rejection by anti-ABO antibodies is not a clinical problem because all graft donors and recipients are selected so that they have the same ABO type. However, less well characterized natural antibodies have become the major barrier to xenotransplantation across species, limiting the use of animal organs for human transplantation.

In the more recent clinical experience, hyperacute rejection of allografts is infrequent. When it does occur, it is usually mediated by antibodies directed against protein alloantigens such as foreign MHC molecules or against an incompletely described alloantigen system expressed on endothelial cells and blood monocytes but not lymphocytes, called E-M antigens. Such antibodies generally arise as a result of prior exposure to alloantigens through blood transfusion, prior transplantation, or multiple pregnancies. These alloantibodies are often of the IgG isotype. By testing recipients for the presence of such antibodies reactive with the cells of potential donors, hyperacute rejection has been virtually eliminated from clinical transplantation.

ACUTE REJECTION

ACUTE HUMORAL REJECTION. Acute humoral rejection is characterized by necrosis of individual cells of the graft blood vessels. The histologic pattern is one of vasculitis (Fig. 16-3) rather than the bland thrombotic occlusion seen in hyperacute rejection. *Acute humoral rejection is often mediated by IgG antibodies against endothelial cell alloantigens (either MHC molecules or E-M antigens) and involves activation of com-*

BOX 16-2. ABO BLOOD GROUP ANTIGENS

The first alloantigen system to be defined was a family of red blood cell surface antigens called ABO. Differences in the ABO system between donors and recipients limit blood transfusions by causing antibody and complement-dependent lysis of the foreign red blood cells. IgM antibodies to these red blood cell antigens pre-exist in a naive host prior to transfusion, and it has been speculated that they arise as responses to cross-reactive microbial antigens. The red blood cell antigen responsible for these transfusion reactions is expressed as a cell surface glycosphingolipid. All normal individuals synthesize a common core glycan, called the O antigen, that is attached to a sphingolipid. A single genetic locus encodes three common alleles of a glycosyl transferase enzyme. The O allele gene product is devoid of enzymatic activity, whereas the A allele gene product transfers a terminal N-acetyl galactosamine moiety and the B allele gene product transfers a terminal galactose moiety. Individuals who are homozygous O cannot attach terminal sugars to the O antigen and express only the O antigen. In contrast, individuals who possess an A allele (AA homozygotes, AO heterozygotes or AB heterozygotes), form the A antigen by adding terminal N-acetyl galactosamine to some of their O antigens. Similarly, individuals who express a B allelle (BB homozygotes, BO heterozygotes, or AB heterozygotes) form the B antigen by adding terminal galactose to some of their O antigens. AB heterozygotes form both A and B antigens from some of their O antigens. Because all individuals express the O antigen, all individuals are tolerant to the O antigen. Individuals with A or B glycosyl transferase alleles are also tolerant to A or B antigens, respectively. However, OO and AO individuals form anti-B IgM antibodies, whereas OO and BO individuals form anti-A IgM antibodies. If a patient receives a transfusion of red blood cells from a donor who expresses a form of the antigen not expressed on self red blood cells, massive red cell lysis will result. It follows that AB individuals can tolerate transfusions from all potential donors and are therefore called universal recipients; similarly, OO individuals can tolerate transfusions only from OO donors but can provide blood to all recipients and are therefore called universal donors. The terminology has been simplified so that OO individuals are said to be blood type O; AA and AO individuals are blood type A; BB and BO individuals are blood type B; and AB individuals are blood type AB.

The same glycosphingolipid that carries the ABO determinants can also be modified by other glycosyl transferases to generate minor blood group differences which elicit milder transfusion reactions. In general, differences in minor blood groups lead to red cell lysis only after repeated transfusions produce a secondary antibody response. Almost all individuals possess a fucosyl transferase that adds a fucose moiety to a side branch of the ABO glycosphingolipid. Upon fucosylation, the O antigen is technically called the H antigen and the whole antigenic system is often called ABH rather than ABO. Addition of fucose moieties at other side branch positions can be catalyzed by different fucosyl transferases and results in epitopes of the Lewis antigen system.

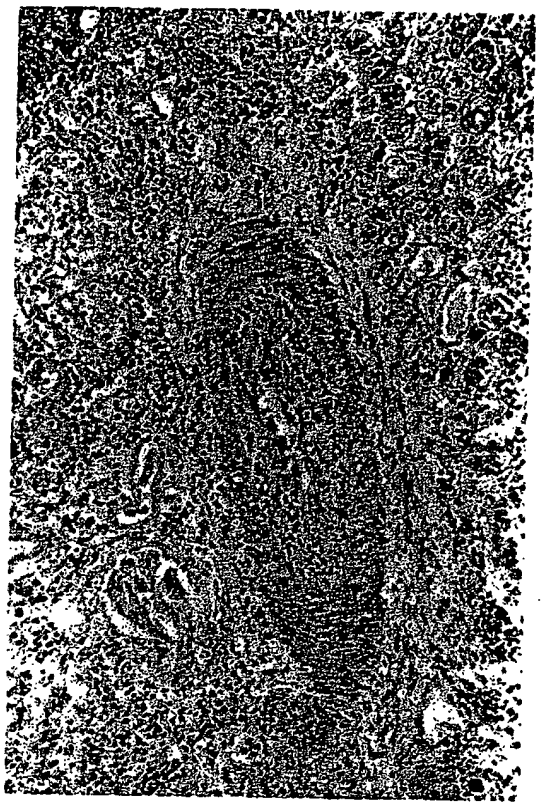

FIGURE 16–3. Acute vascular rejection in the kidney. Antibodies reactive with graft endothelial cells arise in a transplant recipient and cause a destructive inflammatory reaction in the vessel wall. T lymphocytes reactive with graft alloantigens may also participate in vascular injury. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston.)

plement. Because lymphocytes may also be involved, an alternative and more accurate name for this process is **acute vascular rejection.** Lymphocytes may contribute by responding to alloantigens present on vascular endothelial cells, leading to direct lysis of these cells, or they may produce cytokines that activate inflammatory cells, causing endothelial necrosis.

ACUTE CELLULAR REJECTION. This type of rejection is characterized by necrosis of parenchymal cells and is usually accompanied by lymphocyte and macrophage infiltrates (Fig. 16–4). These infiltrating leukocytes are responsible for the lysis of the graft parenchymal cells. *Several different effector mechanisms may be involved in acute cellular rejection including CTL-mediated lysis, activated macrophage-mediated lysis (as in delayed type hypersensitivity [DTH]), and natural killer (NK) cell-mediated lysis* (see Chapter 12). *Several lines of evidence suggest that recognition and lysis of foreign cells by alloreactive CD8⁺ CTLs is probably the most important mechanism in acute cellular rejection.* First, the cellular infiltrates that are present in grafts undergoing acute cellular rejection are markedly enriched for CD8⁺ CTLs specific for graft alloantigens. Second, cloned lines of alloreactive CD8⁺ CTLs can be used to adoptively transfer acute cellular graft rejection. And third, most vascular and parenchymal cells express class I MHC molecules and are susceptible to lysis by CD8⁺ CTLs but are usually resistant to killing by activated macrophages and NK cells.

The identification of both antibody and CTLs as important effector mechanisms in acute graft rejection suggests that this process is similar to normal anti-viral immune responses (see Chapter 15). The basis of this similarity probably arises from the fact that the foreign class I MHC molecules present in the graft are recognized as if they were self MHC molecules associated with endogenously synthesized, e.g., viral, peptides.

CHRONIC REJECTION

Chronic rejection is characterized by fibrosis with loss of normal organ structures (Fig. 16–5). The

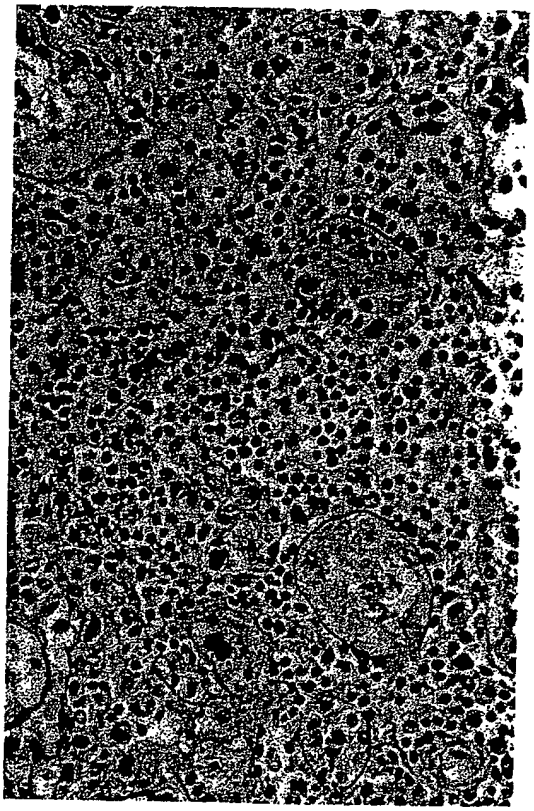

FIGURE 16–4. Acute cellular rejection in the kidney. T lymphocytes reactive with alloantigens in a kidney graft mediate necrosis of tubular epithelial cells as well as of interstitial cells and microvascular endothelial cells. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston.)

pathogenesis of chronic rejection is less well understood than that of acute rejection. The fibrosis of chronic rejection may represent wound healing following the cellular necrosis of acute rejection. However, in many instances, chronic rejection develops without evidence that acute rejection ever occurred. Two other possible explanations of the fibrosis are that chronic rejection represents a form of chronic DTH in which activated macrophages secrete mesenchymal cell growth factors such as platelet-derived growth factor (see Chapter 12) or, alternatively, that chronic rejection is a response to chronic ischemia caused by injury to blood vessels. Vascular injury could result from repeated bouts of antibody-mediated acute humoral rejection, or it may result from cell-mediated injury of microvascular endothelial cells. The stimulus for fibrosis as a result of ischemia is not precisely known.

A variant form of chronic rejection, characterized by proliferation of intimal smooth muscle cells in the walls of muscular arteries, has been described in renal and cardiac transplants (Fig. 16-6). This accelerated arteriosclerosis is the major cause of late graft loss as the involved vessels become completely occluded. In many cases, there is no histologic evidence of prior arterial injury. The smooth muscle cell proliferation in the vascular intima may represent a specialized form of chronic DTH, in which lymphocytes activated by alloantigens in the graft vessel wall induce macrophages to secrete smooth muscle cell growth factors. This is similar to the proposed model of chronic rejection as a form of chronic DTH, except that in the vessel wall smooth muscle cells rather than fibroblasts proliferate and produce collagen.

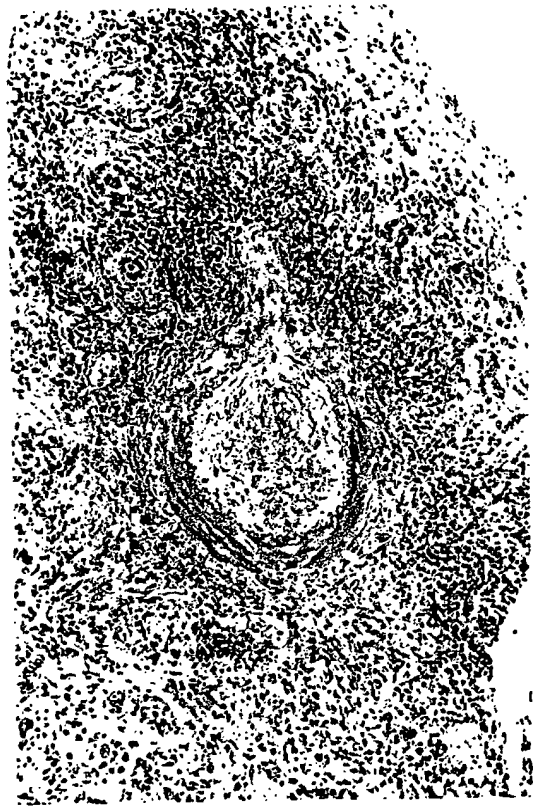

FIGURE 16-5. Chronic rejection in the kidney. *The normal cells of the renal interstitium and tubules are replaced by fibrous tissue. As described in the text, this reaction may represent healing of acute rejection, chronic delayed type hypersensitivity to graft alloantigens, or chronic ischemia. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston.)*

FIGURE 16-6. Transplant associated accelerated arteriosclerosis in the kidney. *In this variant of chronic rejection, the vascular lumen is replaced by accumulation of smooth muscle cells and connective tissue in the vessel intima.*

Prevention and Treatment of Allograft Rejection

If the recipient of an allograft has a fully functional immune system, transplantation almost invariably results in some form of rejection. Two ap-

proaches have been used in clinical practice and in experimental models to avoid or delay rejection:

1. *The graft may be made less immunogenic.*

a. In rodents, as discussed above, this may be accomplished by elimination of passenger leukocytes from grafts, an approach that has not worked in humans and other primates.

b. In human transplantation, the major strategy to reduce graft immunogenicity has been to minimize alloantigenic differences between the donor and recipient by selection. For example, to avoid hyperacute rejection, the ABO blood group antigens of the donor and recipient are always compatible. In addition, MHC molecule allelic differences are also considered at both class I and class II loci. For kidney transplantation, all potential donors and recipients are "tissue-typed" to determine the identity of the HLA molecules that are expressed (Box 16–3). Good matches, involving identity at three or four alleles out of four HLA-A,B loci, are favored. Matching is possible because donor kidneys can be stored in organ banks prior to transplantation until a well-matched recipient can be identified and because, with dialysis, patients needing a kidney allograft can be clinically treated until a well-matched organ is available. In the case of heart and liver transplantation, organ preservation is more difficult and potential recipients are often in critical condition. For these reasons, HLA typing is simply not considered in pairing of potential donors and recipients.

2. *The donor's immune system may be suppressed.* Immunosuppression is the major approach to prevention and management of transplant rejection. Several methods of immunosuppression are commonly used.

a. *Levels of preformed antibodies, such as those that mediate hyperacute rejection, can be reduced by plasmapheresis.* (Plasmapheresis is the removal of blood plasma *ex vivo* and return of washed cells to the body.) Plasmapheresis can also be employed to treat acute humoral rejection, but this has proved to be less successful.

b. *Tolerance to allografts may be induced prior to transplantation by exposure to alloantigens through blood transfusion.* Such transfusions may induce T or B cell tolerance, i.e., may inactivate alloreactive T or B cells, or may stimulate other cells that inhibit alloreactivity.

c. *T cells may be inhibited or lysed by various immunosuppressive treatments. Immunosuppressive drugs are the principal treatment regimen for graft rejection.* Commonly used immunosuppressive therapies include corticosteroids, metabolic toxins, such as azathioprine and cyclophosphamide; irradiation of lymphoid organs; specific immunosuppressive drugs, the prototype of which is cyclosporin A (also known

BOX 16–3. TISSUE TYPING

Tissue typing, also called HLA typing, is the determination of the particular MHC alleles expressed by an individual. The classic approach to HLA typing is to test whether sera collected from certain donors will initiate complement-dependent lysis of an individual's lymphocytes. The sera used for this purpose are obtained from donors who have been many times immunized with foreign cells bearing MHC molecules by transfusion, transplantation, or multiple pregnancies. Such sera characteristically have a low specific antibody titer and react with multiple forms of MHC molecules encoded by several loci. To determine whether an individual expresses HLA-A2, for example, lymphocytes would be tested with a panel of sera, each of which can recognize HLA-A2–bearing cells but may differ in the other specificities they recognize. Only if all of the appropriate sera react and cause lysis is the individual typed as HLA-A2 positive. Naturally, well-characterized human sera are in short supply. Therefore the assays have been adapted to a microscale, where 1 μl of serum plus 1 μl of complement can be tested against 50 target cells in a 2 μl solution in the bottom of a tiny well, kept from evaporating by an overlaid oil drop. In general, tissue typing is still performed this way, using standardized sera that have been tested and characterized by many different laboratories. It is hoped that conventional typing sera will be replaced by monoclonal antibodies reactive with specific MHC molecules. Unfortunately, these reagents are not yet available for most specificities.

The HLA types defined by serologic methods are not necessarily single alleles. Some common HLA types contain several different closely related alleles that may be "split" as new reagents become available that can distinguish among them. Typing with antibodies for class II alleles is especially important. Allogeneic cells often express not only all of the cell that are able to share MHC specificity, the information from secondary MLRs can thus be useful at splitting class II types. Interestingly, some of the cell reactions that are used to split DR types are actually directed against DQ molecules present in linkage disequilibrium with a split of the DR molecule within a type.

Recently, two new approaches have been introduced that should permit more precise typing of the class II loci, replacing both serology and secondary MLRs. The first approach takes advantage of the fact that serologically similar MHC allelic products may be biochemically quite different and can be separated by an analytical technique such as two-dimensional gel electrophoresis combining isoelectric focusing and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (see Box 3–2, Chapter 3). The position of a "spot" on a two-dimensional gel can thus be used to split a serologic type. The second method is even more precise. The polymorphic residues of class II MHC molecules are largely located within exon 2 of both the α and β chains (i.e., within the α1 and β1 polypeptide regions; see Chapter 5). This entire region of the gene can be amplified by polymerase chain reaction (PCR) methods using primers that bind to conserved sequences within the 5′ and 3′ ends of these exons. The amplified segment of DNA can then be readily sequenced. Thus the actual predicted amino acid sequence can be directly determined for the HLA-DR, DQ, and DP alleles of any cell, providing precise molecular tissue typing. Indeed it is exactly for this purpose that the method of polymerase chain reaction was initially developed by Henry Erlich and colleagues.

as cyclosporine); and antibodies reactive with T cell surface molecules.

Corticosteroids have two proposed mechanisms of action. First, they may cause selective lysis of T cells. Corticosteroids are known to cause lysis of immature cortical thymocytes as well as certain T cell lines by activating endogenous nucleases that cleave DNA. However, corticosteroids do not lyse mature medullary thymocytes or mature T cells isolated from blood or peripheral lymphoid organs. A second and more likely proposed mechanism is that corticosteroids act by blocking cytokine gene transcription in and cytokine secretion from mononuclear phagocytes. Inhibition of IL-1, IL-6 and tumor necrosis factor (TNF) synthesis by corticosteroids has been demonstrated both *in vitro* and *in vivo*. Lack of IL-1 and TNF will limit the development of inflammatory reactions. Moreover, IL-1, TNF, and IL-6 may be important costimulators of T cell activation and their relative deficiency may impair specific immunity.

The metabolic toxins in clinical use, namely azathioprine and cyclophosphamide, inhibit the growth of lymphocytes (and other leukocytes) from hematopoietic precursors and may cause preferential lysis of T cells. Irradiation was similarly used as an immunosuppressive agent because T cells are radiosensitive.

The most important immunosuppressive agent in current clinical use is cyclosporin A. Cyclosporin A is a cyclic peptide that is found as a natural metabolite in a species of fungus. The major action of cyclosporin A on T cells is to inhibit transcription of certain genes, most notably the IL-2 gene. Cyclosporin A binds with high affinity to a ubiquitous small molecular size (approximately 12 kilodaltons [kD]) cellular protein called cyclophilin. Cyclophilin has recently been found to have an enzymatic activity that is to catalyze *cis-trans* isomerizations around proline imide bonds in proteins; this peptidyl-prolyl isomerase, or "rotamase," activity serves to catalyze the correct folding of proteins. Cyclosporin A binds to the active site of cyclophilin and blocks catalytic activity. Cyclophilin is not a DNA-binding protein, and it is not known whether or how cyclophilin participates in gene transcription. It is speculated that cyclophilin normally functions to catalyze the proper folding of a positively activating transcription factor and that cyclosporin A blocks this activity. At least two separate DNA-binding proteins that interact with the 5′ regulatory sequences of the IL-2 gene are inhibited in cyclosporin A-treated T cells.

Regardless of its precise mechanism of action, the consequence of cyclosporin A treatment is a profound inhibition of cell-mediated immunity. In the absence of adequate IL-2 and other cytokines, T cells fail to mount an effective immune response. IL-2 is essential for T cell growth and contributes to CTL differentiation. Other T cell genes inhibited by cyclosporin A include *c-myc* and IFN-γ; the failure of these genes to be expressed may also contribute to lack of T cell growth and effector cell activation, respectively.

The introduction of cyclosporin A into clinical practice opened the modern era of transplantation. Prior to the use of cyclosporin A, the majority of transplanted hearts and livers were rejected. Nevertheless, cyclosporin A is not a panacea for transplantation. Drug levels needed for optimal immunosuppression cause kidney damage. For this reason, there is much excitement about a newly characterized fungal metabolite called FK506. FK506 is structurally unrelated to cyclosporin A, but it also binds to a rotamase that catalyzes isomerization about proline imide bonds. The FK506 binding protein, however, is different from cyclophilin. Strikingly, FK506 shares with cyclosporin A the ability to selectively inhibit the transcription of cytokine and certain other genes in T cells. FK506 is active at lower concentrations than cyclosporin A and it may be less toxic. It should be noted, however, that cyclosporin A was also thought to be nontoxic when it was introduced. Nevertheless, FK506, either alone or in combination with cyclosporin A, may expand the scope of clinical transplantation.

Antibodies reactive with T cell surface structures are important agents for treating acute rejection episodes. In the 1960s, commonly used agents for this purpose were polyclonal horse antisera reactive with human lymphocytes or thymocytes. Since the 1980s, mouse monoclonal antibodies to specific T cell surface markers have been more commonly used. The most widely used antibody is OKT3, the first anti-CD3 antibody. It may seem surprising that one would use a potential polyclonal activator such as anti-CD3 to reduce T cell reactivity. *In vivo*, however, OKT3 acts either as a lytic antibody, activating the complement system to eliminate T cells, or opsonizes T cells for phagocytosis. Those T cells that escape probably do so by capping and endocytosing ("modulating") CD3 off their surface, but these may be rendered transiently nonfunctional. Newer antibodies are being tested for immunosuppressive effects without causing T cell elimination. For example, antibodies to the p55 subunit of the IL-2 receptor are in clinical trial because these antibodies can prevent T cell activation by blocking IL-2 binding to activated T cells. The major limitation on the use of mouse monoclonal antibodies is that human recipients rapidly develop anti-mouse Ig antibodies that eliminate the injected mouse Ig. For this reason, attempts are being made to produce human monoclonal antibodies or human-mouse chimeric antibodies that may be less immunogenic.

In addition to these accepted methods of immunosuppression, newer experimental approaches are also in development. One is the use of soluble allogeneic MHC molecules or fragments of MHC molecules to tolerize recipients against specific donor alloantigens. Such peptides are thought to bind competitively to the antigen receptors of alloreactive T cells. A second approach, which has been effective in animal models, is the use of protein toxins, such as diphtheria toxin or ricin A chain, targeted to activated T cells. Targeting can be achieved by conjugating protein toxins to anti-T cell antibodies or by generating fusion proteins. For example, an IL-2-diphtheria

toxin hybrid protein blocks the rejection of allografts in rats by binding to and killing T cells bearing IL-2 receptors. The targeted toxin approach has a theoretical advantage over more conventional monoclonal antibody therapy, in that toxins may mediate more effective elimination of targeted cells, providing more sustained immunosuppression. In addition, a single dose of such toxins may be effective, so that neutralizing host antibodies do not develop. It remains to be seen if toxins, even when targeted, can be used without serious side effects.

Interestingly, in experimental models, if acute allograft rejection is prevented, the graft continues to survive and function even after immunosuppressive therapy is curtailed. It has been postulated that the reason for this is that the recipient becomes tolerant to graft alloantigens or that "suppressor cells" specific for the alloantigens are induced. The relevance of this to human transplantation is uncertain because in humans maintenance immunosuppressive therapy has to be continued permanently.

CLINICAL ORGAN TRANSPLANTATION

We now turn our attention to some of the important clinical issues that have arisen in the practice of solid organ transplantation. Kidney transplants have been successfully performed for the longest period (since the 1950s), and the renal allograft experience has formed the basis of considering transplantation of other organs. For this reason, our discussion will focus on the kidney, but will refer to other organs for comparison when appropriate.

Selection of donor and recipient matches in renal transplantation is based on blood group (ABO) matching, absence of pre-formed antibodies against donor cells in the blood of the recipient (called crossmatching), and human leukocyte antigen (HLA) typing. Analysis of the results of graft survival as a function of HLA type has led to four conclusions:

1. The larger the number of HLA-A and B alleles that are matched between donor and recipient (e.g., three or four of four loci), the better is graft survival, especially in the first year following transplantation. (HLA-C is not routinely matched and is believed to be less important as a target of T cell recognition.)

2. Matches at HLA-DR alleles are important, independent of the number of HLA-A,B matches. Because HLA-DR and DQ are in strong linkage disequilibrium, matching at the DR locus often also matches at the DQ locus. DP typing is not in common use and its importance is unknown.

3. Matching is more predictive of outcome in Europe, where populations are more inbred, than in the United States, where extensive outbreeding has probably diminished linkage disequilibrium among HLA loci.

4. The recipient HLA-DR types influence graft survival independent of the degree of matching. This effect of HLA-DR type of the recipient has been interpreted as an "immune response" gene effect, presumably because host HLA-DR molecules were involved in selecting the mature T cell repertoire. Thus, in recipients who express particular DR alleles, the T cell repertoire may not contain cells specific for some alloantigens, so that grafts bearing these antigens would fail to induce immune responses and would be accepted.

In renal transplantation, immunosuppression with corticosteroids, azathioprine, and anti-T cell antibodies was sufficient to allow survival of unrelated cadaveric donor grafts of 50 to 60 per cent at 1 year and survival of living related donor grafts of 90 per cent at one year. Since cyclosporin A has been introduced, survival of unrelated cadaveric donor grafts has approached about 80 per cent.

In the early years of renal transplantation, rejection was often assessed by biopsy. In the 1970s, biopsy was used less commonly in many transplant centers and rejection severity was usually assessed by following renal function, e.g., as measured by plasma creatinine levels. However, cyclosporin A, which is now universally used in kidney transplantation, is itself a cause of renal injury and can elevate plasma creatinine levels. Thus, a common clinical dilemma is to distinguish declining renal function caused by rejection from that caused by cyclosporin A toxicity. This is usually done by histopathologic examination of biopsy specimens of the transplanted kidney. A useful distinction may be made by examining kidney cells collected by needle biopsy or fine needle aspiration biopsy with an immunocytochemical stain for class II MHC molecule expression. If renal tubular cells express HLA-DR molecules, one may infer that IFN-γ is being produced locally by activated T cells and that renal failure is likely due to rejection. If, on the other hand, HLA-DR is not expressed by tubular cells, the kidney is apparently failing in the absence of local T cell cytokine production and the likely cause is cyclosporin A toxicity. (The functional role or consequence of class II MHC molecule expression on renal tubular cells is unknown.)

Monitoring the rejection of other transplanted organs is somewhat different. Liver allograft rejection can often be measured by assessing liver functions; cyclosporin A is not as toxic to the liver, and failure of bile excretion is a good measure of rejection. Needle biopsies may be used if the clinical pattern is confusing, and expression of HLA-DR molecules by biliary epithelial cells may be indicative of rejection. In the case of heart allografts, functional impairment usually indicates that the rejection process is already quite severe and potentially irreversible. For this reason, cardiac allograft biopsies are performed on regular schedules to assess rejection regardless of cardiac function. (Such biopsy specimens are obtained through a catheter passed into the right ventricle via the venous circulation. Biopsies of the intraventricular septum may be taken with little risk to the patient, since even punctures of the septum will likely scar

without sequelae.) Studies are currently in progress to learn whether these histologic and functional tests may be supplemented or replaced by serologic assays of T cell activation, such as the presence of shed p55 subunit of the IL-2 receptor in the blood.

Acute rejection, when present, is often managed by rapidly intensifying immunosuppressive therapy. This may involve a large "pulse" of corticosteroids or administration of an antibody such as OKT3. Cyclosporin A doses can also be increased. Although acute rejection can cause loss of a graft, most rejection episodes can be reversed by such therapeutic intervention. In modern transplantation, chronic rejection has become the more common cause of allograft failure, especially in cardiac transplantation. Chronic rejection is more insidious than acute rejection, and it is much less reversible. It seems likely that prevention rather than treatment will be the best approach to this problem, but successful intervention will probably require a better understanding of pathogenesis.

Graft survival is dependent upon adequate immunosuppression. This has introduced a clinical dilemma because transplant patients often manifest two other clinical problems caused by immunosuppressive therapy. First, they are particularly susceptible to infections, especially by viruses. Infection by cytomegalovirus, a herpes family virus, is particularly common and may be fatal in the immunosuppressed patient. Second, transplant patients have an increased proclivity to development of certain tumors (see Chapter 17). The three malignancies commonly seen in these patients are B cell lymphomas, squamous cell carcinoma of the skin, and Kaposi's sarcoma. The B cell lymphomas are thought to be sequelae of unchecked infection by Epstein-Barr virus (EBV), another herpes family virus. EBV is a polyclonal activator of B cells, and such polyclonal proliferations appear to predispose to development of monoclonally derived malignant cells (see Chapter 17, Box 17-2). The squamous cell carcinomas of the skin are associated with human papilloma virus and probably also represent virally induced malignancies. Kaposi's sarcoma is now well known for its prevalence in patients with the acquired immunodeficiency syndrome (AIDS) (see Chapter 19) and may be yet another example of a virally induced or provoked malignancy.

In patients receiving immunosuppression for transplantation, clinical problems related to viral infection and virally induced or virally potentiated malignancies are not coincidental. The major thrust of transplant-related immunosuppression is to reduce CTL function, the key effector mechanism of acute cellular rejection. It should thus be no surprise that defense against viruses, the physiologic function of CTLs, is preferentially undermined.

BONE MARROW TRANSPLANTATION

Bone marrow transplantation is really the transplantation of pluripotent hematopoietic stem cells (see Chapter 2). Upon transplantation, these cells then repopulate the recipient's bone marrow with their differentiating progeny. Clinically, allogeneic bone marrow transplantation may be used to remedy acquired defects in the hematopoietic system or in the immune system, since both types of cells develop from a common stem cell. It has also been proposed as a means of correcting inherited enzyme deficiencies, by providing a self-renewing source of enzyme-producing cells. In addition, allogeneic bone marrow transplantation may be used as part of the treatment of bone marrow malignancies, i.e., leukemias. In this case, the chemotherapeutic agents needed to destroy leukemia cells also destroy normal marrow elements and bone marrow transplantation is used to "rescue" the patient from the side effects of chemotherapy. For other malignancies, when the marrow is not involved by tumor or when it can be purged of tumor cells, the patient's own bone marrow may be harvested and reinfused after chemotherapy. This procedure, called autologous bone marrow transplantation, lacks the immunologic problems associated with allogeneic bone marrow transplantation and will not be discussed further.

There are several unique problems associated with allogeneic bone marrow transplantation that lead us to consider it separately from solid organ transplantation:

1. The transplanted stem cells must "home" to establish themselves in the appropriate environment; surgeons cannot "place" the stem cells in a particular location in the bone marrow. Moreover, experimental and clinical experience suggests that there are only a limited number of "niches" within marrow cavities and if these are occupied at the time of transplantation, the graft cells cannot establish themselves. The recipient often must be "prepared" with intense radiation and chemotherapy prior to transplantation to deplete his or her own marrow cells and vacate these sites.

2. Allogeneic stem cells are readily rejected by even a minimally immunocompetent host. The mechanisms of rejection are not completely known, but it has been suggested that in addition to specific immune mechanisms, hematopoietic stem cells may also be rejected by NK cells. The recipient's immune system must be nearly ablated to permit successful bone marrow transplantation. Again, this is accomplished by intense "preparation" of the recipient with radiation and chemotherapy.

3. Graft cells may mount a rejection response against the host. This response, called the **graft-versus-host reaction,** can injure the host and cause graft-versus-host disease (GVHD) (see below). The graft-versus-host reaction arises only in the setting of extreme injury to the host immune system, a consequence of the preparation necessary to avoid stem cell rejection.

4. Recipients of allogeneic bone marrow transplants often show prolonged and profound immunodeficiencies. In human bone marrow transplantation, this is a major cause of morbidity and mortality.

Graft-versus-Host Disease

Graft-versus-host disease is the principal limitation on the use of bone marrow transplantation. As in solid organ transplantation, GVHD may be classified on the basis of histologic patterns into acute and chronic categories.

Acute GVHD involves epithelial cell necrosis in three principal target organs, skin, liver, and the gastrointestinal tract (Fig. 16-7). In the liver, the biliary epithelial cells but not the hepatocytes are involved. Clinically, acute GVHD is characterized by skin rash, jaundice, and diarrhea. When the epithelial necrosis is extensive, the skin or lining of the gut may simply slough off. In this circumstance, acute GVHD may be fatal.

Chronic GVHD is characterized by fibrosis and atrophy of one or more of the same organs, without evidence of acute cell necrosis (Fig. 16-7). On occasion, necrosis and fibrosis can be present at the same time, leading to a diagnosis of acute and chronic GVHD. When severe, chronic GVHD leads to complete dysfunction of the affected organ and may also be fatal.

In animal models, acute GVHD is initiated by mature T cells present in the bone marrow inoculum. Elimination of mature donor T cells from the graft can prevent development of GVHD. Efforts to eliminate T cells from human marrow inoculum have reduced the incidence of GVHD, but also appear to reduce the efficiency of engraftment; mature T cells, perhaps through production of IL-3 and other colony-stimulating factors (CSFs), significantly improve stem cell repopulation. Since failure to engraft is even more lethal than GVHD, it is not yet clear whether the removal of T cells will be clinically beneficial. An alternative approach in current clinical trial is to combine removal of T cells with supplemental IL-3 and/or granulocyte-macrophage colony-stimulating factor (GM-CSF) to promote engraftment.

Although GVHD is initiated by T cell recognition of host alloantigens, the effector cells that produce epithelial cell necrosis are less well defined. Histologically, NK cells are often attached to the dying epithelial cells, suggesting that NK cells are the effector cells of acute GVHD. This has raised the issue of how NK cells lyse normal epithelial cells, since they do not recognize alloantigens and cannot lyse epithelial cells *in vitro*. It has been proposed that the NK cells are activated by locally produced IL-2 to differentiate into lymphokine-activated killer (LAK) cells. As we

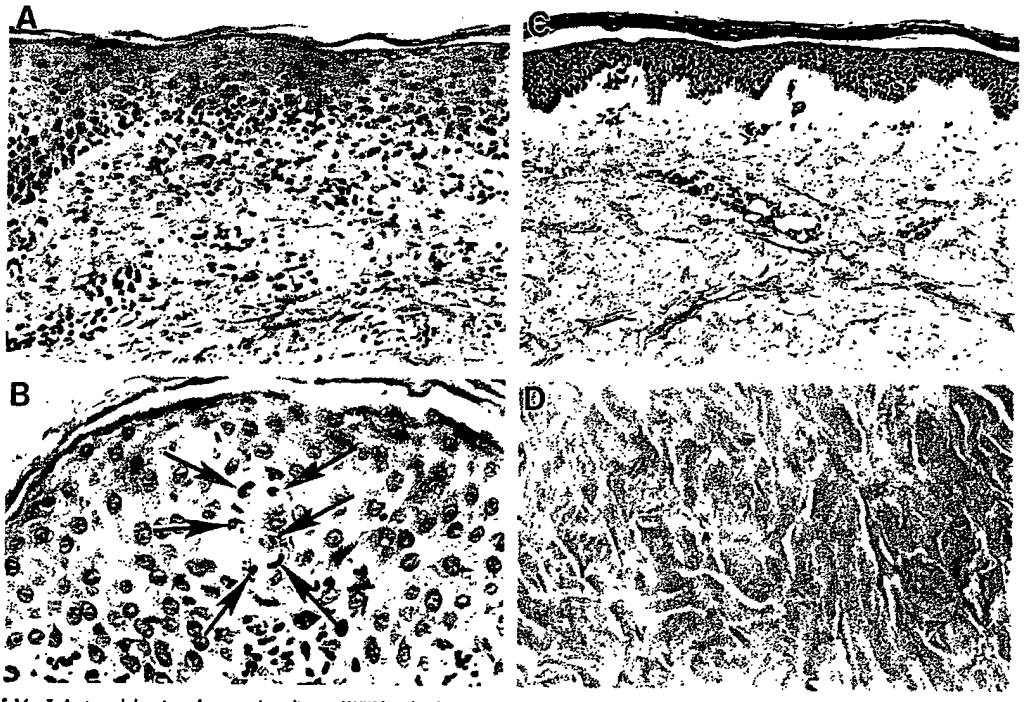

FIGURE 16-7. Acute and chronic graft-versus-host disease (GVHD) in the skin. At low magnification, acute GVHD appears as a sparse lymphocytic infiltrate at the dermal-epidermal junction (A); *at higher magnification* (B), *lymphocytes can be identified in the epidermis adjacent to injured epithelial cells* (arrows). *In contrast, chronic GVHD* (C) *shows fibrosis of the dermis and epidermal thinning. At higher magnification* (D), *dermal appendages can be seen to be trapped in the dense fibrosis. (Courtesy of Dr. George Murphy, Departments of Dermatology and Pathology, University of Pennsylvania, Philadelphia.)*

discussed in Chapter 12, LAK cells can lyse normal cell types, including epithelium, and are not restricted by MHC molecules.

The relationship of chronic GVHD to acute GVHD is unknown and raises issues similar to those of relating chronic allograft rejection to acute allograft rejection. For example, chronic GVHD may represent the fibrosis of wound healing secondary to epithelial cell necrosis. However, chronic GVHD can arise without evidence of prior acute GVHD. An alternative explanation is that chronic GVHD may represent a response to ischemia caused by vascular injury.

Both acute and chronic GVHD are commonly treated with intense immunosuppression. It is not clear that either condition responds very well. A possible explanation is that conventional immunosuppression is targeted against T lymphocytes, especially CTLs. This works well in allogeneic rejection of solid organs, but is less efficacious for NK cell-mediated or LAK cell-mediated responses. Much effort has focused on prevention of GVHD. HLA typing is very important for preventing GVHD. Indeed, most human bone marrow transplants are performed between siblings who are completely identical at all HLA loci and clinical GVHD is due to differences at minor histocompatibility loci. Transplantation between parent and child may be performed when necessary, but only with stringent elimination of mature T cells.

Immunodeficiency Following Bone Marrow Transplantation

As noted above, bone marrow transplantation is often accompanied by clinical immunodeficiency. Several factors may contribute to defective immune responses in recipients:

1. Bone marrow transplant recipients may be unable to regenerate a completely new T cell repertoire. The transplanted bone marrow may not contain a sufficient number and variety of self-renewing lymphoid progenitors, and the thymus gland of the recipient may have undergone irreversible changes during or after involution in early childhood.

2. The ablation of the specific immune system in preparation for bone marrow transplantation unmasks a "natural suppression" system that prevents adequate regeneration of a specific immune system. Some immunologists have referred to specific populations of "natural suppressor" cells, observed after whole body irradiation. Such natural suppressor cells may be identical or related in lineage to NK cells.

3. The allogeneic host environment may overwhelm the developing immune system with alloantigenic stimuli that prevent development of a normal repertoire. An alternative statement of this explanation is that the graft-versus-host reaction pre-empts normal immunity. Many immunologists regard immunodeficiency as part of GVHD. However, immunodeficiency may well exist in bone marrow transplant recipients who lack clinically overt or histologically detectable GVHD.

The consequence of immunodeficiency is that bone marrow transplant recipients are very susceptible to viral infections, especially with cytomegalovirus. They are also susceptible to EBV-provoked B cell lymphomas. However, there has not been an increased incidence of other malignancies, namely squamous cell carcinoma of the skin and Kaposi's sarcoma, noted in solid organ transplant recipients. The basis for this difference is unclear.

SUMMARY

Transplantation of tissues from one individual to a genetically nonidentical recipient leads to a specific immune response, called rejection, that can destroy the graft. The major molecular targets in transplant rejection are non-self allelic forms of class I and class II MHC molecules.

The reaction to foreign class I and class II molecules can be analyzed *in vitro* in the MLR. In general, foreign class I molecules stimulate alloreactive $CD8^+$ CTLs whereas foreign class II molecules stimulate alloreactive $CD4^+$ helper T lymphocytes, although the largest reactions occur when there are differences at both class I and class II loci.

In vivo, rejection is mediated by antibodies and by CTLs. Help, provided by $CD4^+$ T cells, is often necessary to initiate the rejection reaction. Alloreactive $CD4^+$ T cells may be stimulated by foreign class II MHC-positive APCs in the graft.

Several patterns of rejection can occur in solid organ transplants. Pre-existing antibodies, often IgM directed against ABO antigens on endothelial cells, can cause hyperacute rejection characterized by thrombosis of graft vessels. Antibodies produced in response to the graft cause blood vessel cell necrosis, called acute humoral or vascular rejection. Infiltrating alloreactive CTLs cause parenchymal cell necrosis, called acute cellular rejection. Chronic rejection, characterized by fibrosis, may represent healing of acute rejection or may represent a chronic delayed type hypersensitivity reaction. A variant of chronic rejection in the walls of muscular arteries can produce accelerated arteriosclerosis and ischemic injury of the graft.

Rejection may be minimized by reducing the immunogenicity of the graft, often by limiting the number of differences between donor and recipient MHC alleles. Rejection is both prevented and treated by immunosuppression. The most commonly used immunosuppressive drugs are agents that reduce T cell function and number. Such agents include corticosteroids; cytotoxic drugs with specificity for T cells, such as azathioprine; immunosuppressive drugs, such as cyclosporin A; and antibodies directed against T cell surface molecules.

Patients receiving solid organ transplants may

experience complications related to their therapy. These include viral infections, especially with cytomegalovirus, and virus-related malignancies, such as B cell lymphoma, squamous cell carcinoma of the skin, and Kaposi's sarcoma.

Bone marrow transplant recipients are very susceptible to graft rejection and require intense preparatory immunosuppression. In addition, two unique problems not seen with solid organ transplants may develop. First, lymphocytes in the bone marrow graft may respond to alloantigens of the host, producing GVHD. Acute GVHD is characterized by epithelial cell necrosis in the skin, liver, and gut, causing a rash, jaundice, and diarrhea, respectively. When severe, acute GVHD may be fatal. Chronic GVHD is characterized by fibrosis and atrophy of one or more of these same target organs and may also be fatal. Second, bone marrow transplant recipients often have immunodeficiencies, rendering them susceptible to infections.

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Exhibit 2-3

CHAPTER SEVENTEEN

IMMUNITY TO TUMORS

Malignant tumors, or cancers, grow in an uncontrolled manner, invade normal tissues, and often metastasize and grow at sites distant from the tissue of origin. In general, cancers are derived from one or only a few normal cells that have undergone a poorly understood process called malignant transformation. Cancers can arise from almost any tissue in the body. Those derived from epithelial cells, called carcinomas, are the most common kinds of cancers. Sarcomas are malignant tumors of mesenchymal tissues, arising from cells such as fibroblasts, muscle cells, and fat cells. Solid malignant tumors of lymphoid tissues are called lymphomas, and marrow and blood-borne malignant tumors of lymphocytes and other hematopoietic cells are called leukemias.

Hypothetically, a major function of the immune system could be to recognize and destroy spontaneously arising malignantly transformed cells, so-called "mutant clones," before they grow into tumors. This idea, called **immunosurveillance**, was articulated by Macfarlane Burnet and Lewis Thomas in the 1950s and 1960s. In addition, immune responses to malignant cells may be protective even after these cells have grown into tumors. If malignant cells and tumors can stimulate immune responses, it follows that they must express **tumor antigens** that are recognized as foreign by the tumor-bearing host. Furthermore, if the concept of immunosurveillance is valid, immune effector cells, such as B cells, helper T cells, cytolytic T lymphocytes (CTLs), or natural killer (NK) cells must be able to recognize tumor antigens and mediate the killing of tumor cells.

A common histologic observation which suggests that tumors may be immunogenic is the presence of mononuclear cell infiltrates, composed of T cells, NK cells, and macrophages, surrounding many tumors. Although such infiltrates may often result after tissue destruction caused by the tumor, they are more frequently present around certain types of tumors, including testicular seminomas, thymomas, medullary breast carcinoma, and malignant melanomas in the skin, irrespective of the presence of other inflammatory stimuli such as infection or tissue necrosis. In fact, the presence of lymphocytic infiltrates in medullary breast carcinomas and malignant melanomas is associated with a better prognosis compared with histologically similar tumors without infiltrates. Another histopathologic indication that tumors stimulate immune responses is the frequent finding of lymphocytic proliferation (hyperplasia) in lymph node draining sites of tumor growth. Furthermore, there is often evidence of cytokine effects in tumors, such as class II MHC expression on tumor cells and endothelial cells of tumor vessels, suggesting an active immune response at the sites of the tumors.

At one time, the major effector mechanism for tumor immunosurveillance was considered to be the CTL. In fact, when CTLs were first discovered, their only demonstrated function was the artificial role of killing allogeneic cells in a transplant or a mixed leukocyte reaction (MLR), and antitumor activity was the assumed physiologic role for these cells. More recently, we have come to appreciate that CTLs are most important in anti-viral immunity. Furthermore, a critical evaluation of the immunosurveillance hypothesis suggests that it is not generally valid for most forms of cancer. For example, if the immune system is required to prevent the frequent occurrence of cancers, one would expect that many more malignant tumors would develop in individuals with congenital or acquired immunodeficiencies than in immunocompetent individuals. In fact, this is not the case for most common forms of cancers, such as carcinomas of the colon, lung, or breast. There is, however, a strikingly increased incidence of certain forms of cancer in immunosuppressed individuals, and many of these cancers may result from infections with tumor-causing viruses (as discussed later in the chapter). Thus, the concept of immunosurveillance may be most relevant for the limited subset of cancers caused by oncogenic viruses.

The idea that the immune system responds to tumors has served as the stimulus for a branch of immunology called **tumor immunology.** The field of tumor immunology encompasses the study of specific acquired immune responses to tumors, the antigens on tumor cells which induce immune responses, immunologic effector mechanisms that kill tumor cells, and immunologic approaches for detecting, diagnosing and treating cancers. The great progress we have made over the last decade in understanding the physiology of normal immune responses is already being applied to the important practical problems of prevention and treatment of tumors. This chapter discusses these different aspects of tumor immunology, referring to the basic principles of the cognitive and effector arms of the immune response which have already been described in detail in previous chapters.

TUMOR ANTIGENS

The abnormal growth behaviors of malignant tumors are the reflection of complex abnormalities in physiology which result from expression of mutated or viral genes and/or deregulated expression of normal genes. It is a reasonable assumption, therefore, that cancer cells express certain proteins that are either not expressed at all or are present in much lower quantities in normal cells. Such proteins may be the tumor antigens, that are seen as foreign by a tumor host, resulting in specific immune responses to tumor cells. In addition, surface proteins peculiar to tumors may serve as targets for effectors of natural immunity, such as NK cells.

The fact that tumor cells express antigens that can stimulate immune responses in the host has been clearly demonstrated in both experimental animal models and in human cancer patients. Two main approaches have been used to identify tumor antigens. First, antibodies can be produced by immunizing an animal with the tumor cells, and these antibodies can then be used as probes for different molecules expressed on the tumor cell surface. Second, tumor anti-

gens can be operationally defined as molecules that stimulate T cell-mediated rejection of tumor transplants in an animal previously immunized with the tumor. The tumor antigens that are defined by rejection experiments are called **tumor-specific transplantation antigens** (TSTAs); they include tumor cell proteins that have been processed and presented by the tumor cell in association with major histocompatibility complex (MHC) molecules. Some tumor antigens are unique to particular tumors, whereas others are present on many or all cancers of a specific type.

A fundamental, and as yet unanswered, challenge in the field of tumor immunology is to determine the role that immune responses to tumor antigens play in the control of naturally occurring tumors. For example, there is no compelling evidence that the presence of anti-tumor antibodies serves to block tumor growth. It is also clear that immune responses to TSTAs often do not block the outgrowth of artificially induced tumors and may be effective only in the experimental situation of tumor transplantation into a specifically immunized animal. It should be kept in mind, however, that our difficulty in demonstrating the physiologic significance of tumor antigens may be a reflection of our limited (but improving) ability to analyze the complex biology of tumor growth and *in vivo* immune responses.

This portion of the chapter describes several types of tumor antigens that have been studied in some detail, providing various insights into the biology of tumor-host interactions.

Unique Tumor Antigens

Studies in the 1950s of chemical-induced or radiation-induced tumors in inbred strains of mice demonstrated the existence of antigens expressed exclusively by the cells of one individual tumor. Although analogous unique tumor antigens have not been demonstrated in naturally occurring human tumors, this model of tumor antigens is significant because it is the clearest demonstration that the immune system can specifically prevent the growth of malignant tumors. In a typical study of this sort, a sarcoma is induced in an inbred mouse by painting its skin with the chemical carcinogen, methylcholanthrene (MCA). These MCA-induced sarcomas can be excised from the original host mouse and introduced into other mice or back into the original animal. Upon transplantation of these tumors into other syngeneic mice, the tumors grow and eventually kill the new host. In contrast, reintroduction of the tumor into the original host results in a specific immunologic rejection of the tumor. Adoptive transfer experiments show that rejection is mediated mainly by tumor-specific CTLs. Alternatively, the cells of a tumor from one mouse can be killed by irradiation and used to immunize a second syngeneic mouse. Subsequent introduction of live cells from the original tumor into the immunized mouse results in immunologic rejection of the tumor transplant (Fig. 17-1). These experiments demonstrate that the *rejection of the transplanted tumors displays two cardinal features of specific immune responses, namely specificity and memory.* In addition, they suggest that CTLs are an important effector mechanism for anti-tumor immunity. Since the tumor antigens in this experimental system are defined on the basis of rejection of transplanted tumor cells, they are called TSTAs. A remarkable property of these TSTAs is their enormous diversity, reflected by the specificity of the immune responses to each individual tumor. For example, one MCA-induced sarcoma does not induce protective immunity against another MCA-induced sarcoma, even if both tumors are derived from the same mouse (Table 17-1, experiment 1).

Although TSTAs were described over 30 years ago, attempts to define their molecular nature were for a long time largely unsuccessful. For many years, investigators tried, and failed, to raise monoclonal antibodies specific for these antigens. Recently, however, genes encoding some of these TSTAs have been identified. The strategy used was to artificially mutagenize a tumorigenic (tum^+) mouse cell line and isolate non-tumorigenic (tum^-) variant cell lines. It was established that the tum^- phenotype was due to the

TABLE 17-1. Transplantation Antigens on Chemically and Virally Induced Tumors

Experiment	Treatment of Mouse: *Immunization with Killed Tumor Cells from*	Treatment of Mouse: *Challenge with Live Tumor Cells from*	Result	Conclusion
1	Chemically induced sarcoma A	Chemically induced sarcoma A	No growth	Immunity to chemically induced tumors is specific for individual tumors.
	Chemically induced sarcoma A	Chemically induced sarcoma B	Growth of chemically induced sarcoma B	
2	MSV-induced sarcoma A	MSV-induced sarcoma A	No growth	Immunity to virus-induced tumors is virus-specific.
	MSV-induced sarcoma A	MSV-induced sarcoma B	No growth of sarcoma B	
	MSV-induced sarcoma A	Chemically induced sarcoma C	Growth of chemically induced sarcoma C	
	MSV-induced sarcoma A	MuLV-induced sarcoma D	Growth of MuLV-induced sarcoma D	

Abbreviations: MSV, murine sarcoma virus; MuLV, murine leukemia virus.

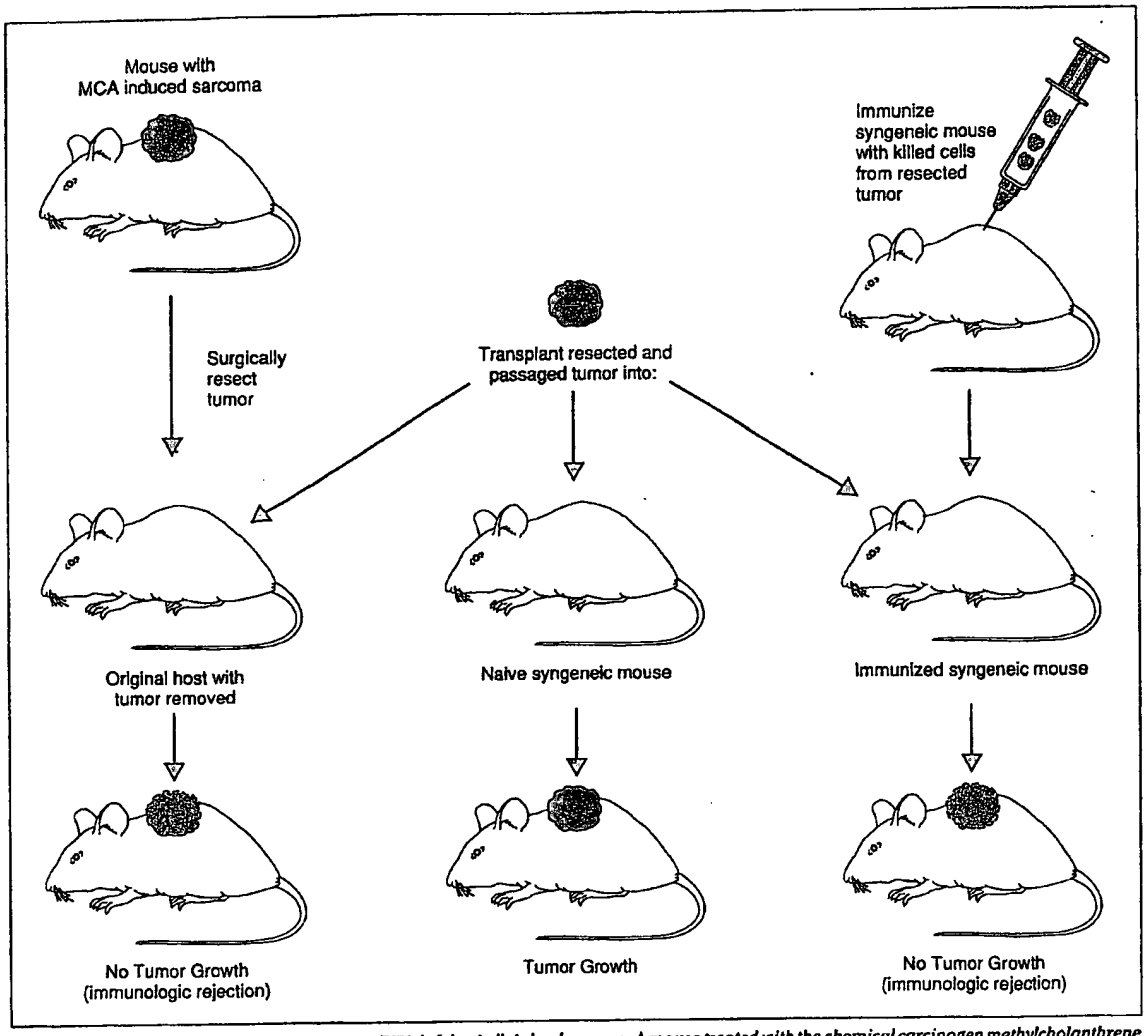


FIGURE 17-1. Tumor-specific transplantation antigens (TSTAs) of chemically induced sarcomas. A mouse treated with the chemical carcinogen methylcholanthrene (MCA) develops a sarcoma. If this tumor is resected and transplanted into a normal syngeneic mouse, the tumor will grow. In contrast, the original tumor-bearing animal that was cured by surgical resection will reject a subsequent transplant of the same tumor. Injection of killed cells from the same tumor into a syngeneic mouse induces the same type of protective immunity.

presence of unique TSTAs that were not present on the parent tum^+ cells. These TSTAs stimulated a specific CTL-mediated rejection of transplanted tum^- cells. In other words, the tum^- variant expresses TSTAs and is immunogenic whereas the tum^+, TSTA-negative parent line is not immunogenic. A cosmid library of genes was derived from the tum^- line, and these genes were then transfected into the tum^+ line. By this approach, genes were identified that encoded the TSTAs and that would convert the cells to a nontumorigenic, i.e., immunogenic, phenotype (Fig. 17-2). In this system, the TSTA-encoding genes are highly diverse and apparently represent point mutations of various unrelated normal cellular genes. It is plausible that a similarly diverse group of mutated genes code for the unique TSTAs in the MCA-sarcoma model described above.

The proteins produced by such tumor-specific genes are endogenously synthesized, processed, and presented to the host immune system, usually in association with class I MHC molecules. The processing and presentation of endogenously synthesized proteins presumably occurs normally in many or all cells. If the proteins are normal self proteins, they do not induce immune responses because of the absence of self reactive T cells. If, however, the proteins are altered or mutated forms of normal proteins, they will be recognized by specific CTLs and will serve as targets for cell lysis and rejection. Thus, *in different tumors, the TSTAs may be altered forms of different cellu-*

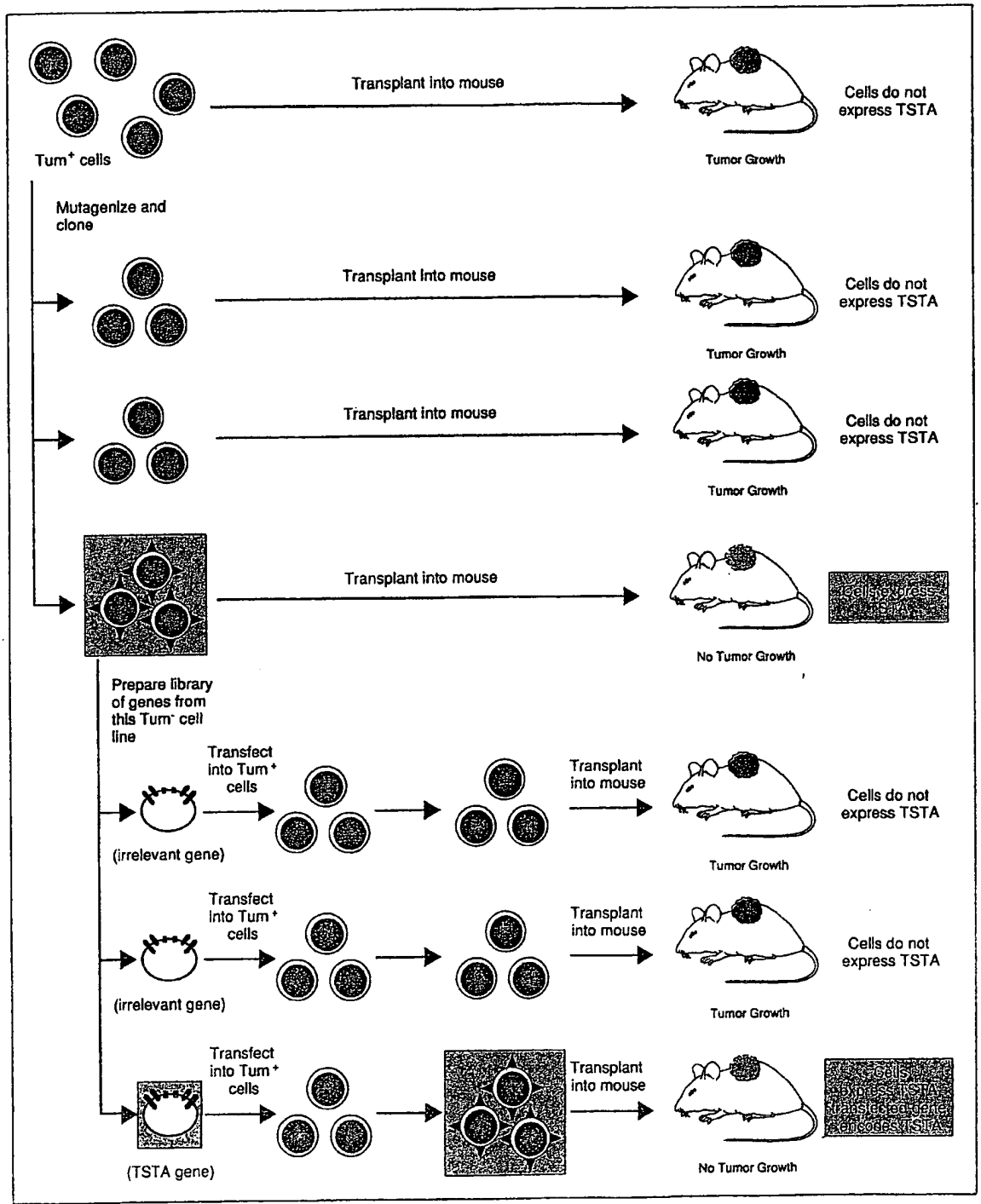


FIGURE 17-2. Identification of genes encoding tumor-specific transplantation antigens (TSTAs). Cells expressing TSTAs and genes encoding TSTAs are shown in shaded boxes. The genes identified by this approach encode a variety of apparently unrelated cellular proteins, with point mutations resulting in one or a few amino acid differences from normal proteins. These mutated proteins induce immune responses that result in tumor transplant rejection.

lar proteins. The fact that most of these unique TSTAs are only present on the cell surface in the form of peptides bound to MHC molecules is probably why it has been difficult to raise antibodies against them. The relationship (if any) of the mutations to the malignant phenotype of the tumor cells is not known. The mutations may be a result of the high doses of mutagens or carcinogens used to generate the experimental tumors and tumor variants. A similar rate of mutations and the resultant expression of unique tumor antigens may not occur in human tumors, since human tumors are rarely caused by such high doses of mutagens.

Antigens Shared by Different Tumors

Most tumor antigens studied are not unique to individual tumors, but are shared by different tumors. Furthermore, most, if not all, of these diverse groups of antigens may be found on normal cells or benign tumor cells. Such antigens are often called **tumor-associated antigens** (TAAs). There are several classes of these antigens, and many different ones may be expressed on the same tumor. An example of the diversity of TAAs is seen in the phenotype of human melanomas (Box 17-1).

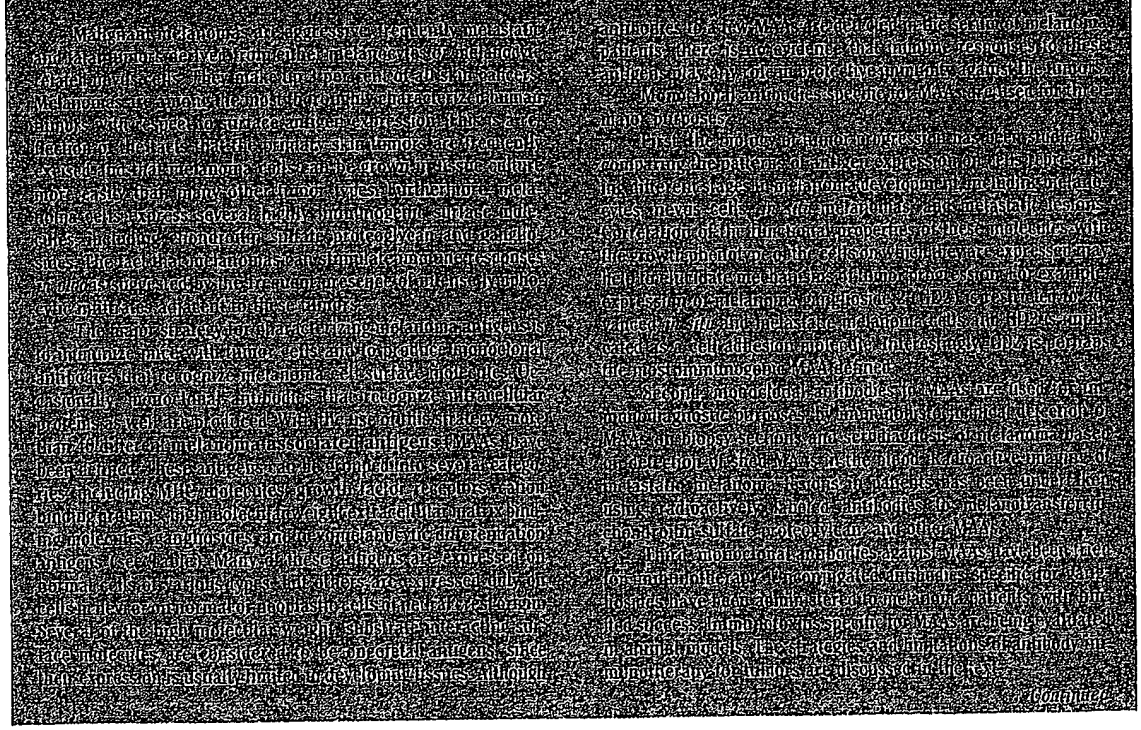
Silent Genes

Some cellular genes that are not normally expressed in an individual may become transcriptionally active in tumor cells, and therefore their protein products may be recognized by the host as foreign antigens. These genes are called "silent" genes. For example, the non-polymorphic class I MHC-like molecule called the thymic leukemia antigen (Tla) is not normally expressed at any time in some strains of mice, whereas other strains express the antigen on developing thymocytes. T cell leukemias from any strain of mice invariably express Tla molecules, and these antigens can elicit antibody responses in normally Tla-negative mice. Tla however, does not appear to elicit tumor rejection responses, and its role in protective immunity is not known. Human counterparts of tumor-associated silent gene products have not yet been described.

Oncofetal Antigens

Oncofetal antigens make up another class of tumor-associated antigens. They are normally ex-

BOX 17-1. MELANOMA ANTIGENS



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Melanoma-Associated Antigens

Category	Example	Biochemical Characteristics	Significant Features
High molecular weight substrate interacting antigens	Chondroitin sulfate proteoglycan	>400 kD; 250 kD polypeptide core	Expressed on membrane spikes; involved in intercellular adhesion, and matrix attachment; highly immunogenic
	Melanoma-associated cellular adhesion molecule	105 and 130 kD	Role in matrix adhesion
	Placental membrane antigen	120 and 94 kD	Role in matrix adhesion
	High molecular weight proteins with ganglioside-like distribution pattern	260 kD	Role in matrix adhesion; highly specific to melanomas
Gangliosides	GD2 9-O-acetylated GD3 GD3 GM2	Gangliosides	Expressed in brain and tumors of neural crest origin only; implicated in cell adhesion; GD2 and GD3 expression characteristic of advanced or metastatic lesions; highly immunogenic
Growth factor receptors	Epidermal growth factor receptor (EGF-R)		Expressed on advanced tumors; EGF is mitogenic for melanoma cells *in vitro*
	Nerve growth factor receptor (NGF-R) Insulin growth factor receptor Platelet-derived growth factor receptor (PDGF-R) Transforming growth factor β receptor (TGF-β-R)		Expressed on all cultured melanoma cells
Cation transport and binding proteins	Melanotransferrin	97 kD monomeric sialoglycoprotein; related to transferrin	Expressed on all cultured melanoma cells; highly immunogenic
	Calcium-binding S-100	21 kD highly acidic cytoplasmic protein	Member of calcium-binding protein family; expressed by neural crest-derived tumors and normal tissues; widely used for immunohistochemical diagnosis of nonpigmented melanomas.
Class II MHC	HLA-DR		Expressed on many primary tumor explants; no correlation with behavior *in vivo*
ICAM-1/2	—	90 kD	Ligands for LFA-1
Pigmentation-associated antigen		70-80 kD	Found in melanosomes of pigmented normal and malignant melanocytes
Differentiation antigens	Nevus antigen Gangliosides Galactocerebrosides Myelin-associated glycoprotein Others	Variable	Antigens on melanoma cells which correspond to antigens expressed on normal nevomelanocytes

Abbreviations: HLA, human leukocyte antigen; kD, kilodalton; MHC, major histocompatibility complex; GD, ganglioside; ICAM-1/2, intercellular adhesion molecule-1/2; LFA-1, leukocyte function-associated antigen-1.

pressed on developing (fetal) but not adult tissues. The expression of these proteins on tumor cells is the result of the derepression of genes by unknown mechanisms. As techniques for detecting these antigens have improved in sensitivity, it has become clear that their expression in adults is not strictly limited to tumors. These proteins are found in tissues in various inflammatory conditions, and even in small quantities in normal tissues. Furthermore, oncofetal antigens are not antigenic in the host, since they are expressed as self proteins during development. Not surprisingly, therefore, no evidence exists indicating that an individual mounts an immune response to these antigens on tumor cells. Nonetheless, the study of oncofetal antigens is useful for diagnostic purposes and provides some insights into tumor biology. The two most thoroughly described oncofetal antigens are **alpha-fetoprotein** (AFP), and **carcinoembryonic antigen** (CEA).

AFP is a 70 kilodalton (kD) α-globulin glycopro-

tein normally synthesized and secreted in fetal life by the yolk sac and liver. Fetal serum concentrations can be as high as 2 to 3 mg/ml, but in adult life the protein is replaced by albumin and only low levels are present in the serum. Serum levels of AFP can be significantly elevated in patients with hepatocellular carcinoma, germ cell tumors, and occasionally gastric and pancreatic cancers. Elevated serum AFP is a useful indicator of advanced liver or germ cell tumors or of recurrence of these tumors after treatment. Furthermore, the detection of AFP in tissue sections by immunohistochemical techniques can help in the pathologic identification of tumor cells. The diagnostic value of AFP as a tumor marker is limited by the fact that elevated serum levels are also found in non-neoplastic liver diseases such as cirrhosis.

CEA is a highly glycosylated 180 kD integral membrane protein that is a member of the Ig gene superfamily. CEA is also released into the extracellular fluid. Normally, high CEA expression is restricted to the gut, pancreas, and liver during the first two trimesters of gestation and reduced expression is found in normal adult colonic mucosa and lactating breast. CEA expression is greatly increased in colonic carcinomas, resulting in a rise in serum levels. Assays for serum CEA are used to monitor the spread of colon carcinoma or its recurrence after primary treatment. Recent studies have demonstrated that CEA functions as an intercellular adhesion molecule, promoting CEA-expressing cells to bind to one another. Thus, CEA may play a role in the way tumor cells interact with one another and with the tissues into which they are growing.

ANTIGENS ENCODED BY GENOMES OF ONCOGENIC VIRUSES

Viral antigens represent the most immunogenic molecules on malignant tumors and may be the most significant type of tumor antigens for protective tumor immunity. Both RNA and DNA viruses are implicated in the development of tumors in both experimental animals and humans. Virally induced tumors usually contain integrated proviral genomes in their cellular genomes and often express viral genome-encoded proteins. These endogenously synthesized proteins can be processed, and complexes of processed viral peptides with MHC molecules (usually class I) may be expressed on the tumor cell surfaces. Thus, tumor cells expressing viral proteins can stimulate and/or become the targets of specific T cell immune responses. Structurally and biologically distinct antigens are produced by various DNA and RNA tumor viruses.

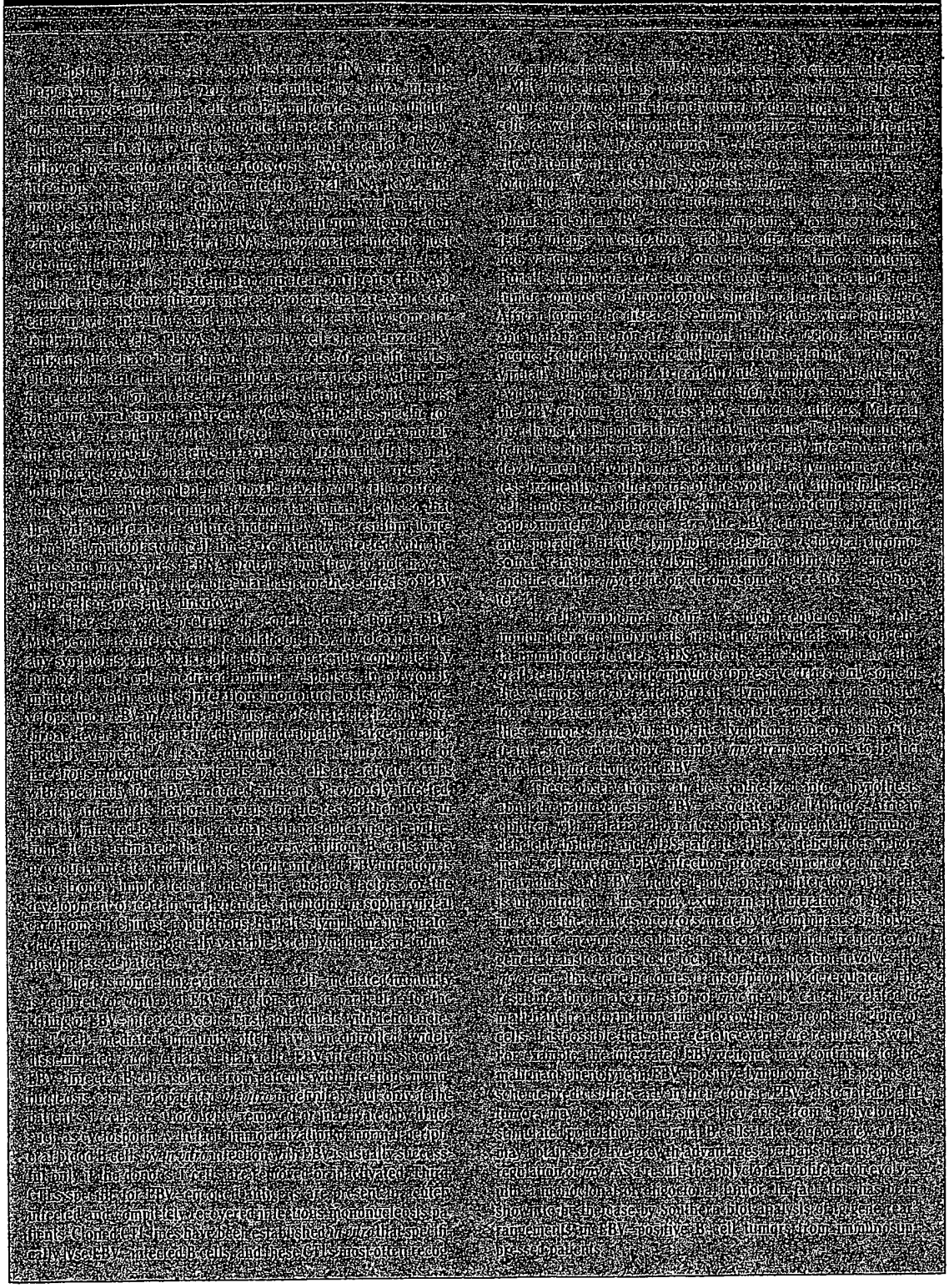
DNA viruses are probably involved in the development of several different tumors. The papova viruses (including polyoma virus and SV40) and the adenoviruses induce a variety of malignant tumors in neonatal or immunodeficient adult rodents. Several different genes in these viruses cooperate to cause malignant transformation of infected cells. In humans, DNA viruses are associated with the development of several different tumor types. Examples include the association between Epstein-Barr virus (EBV) and B cell lymphomas (Box 17-2), human papilloma virus (HPV) and cervical carcinoma, and hepatitis B virus (HBV) and hepatocellular carcinoma. The viral genes responsible for producing the malignant phenotype in these human tumors are not well defined.

In most cases, DNA virus-induced tumor cells do not produce viral particles, and virally encoded protein antigens that are not components of infectious viral particles may be found in the nucleus, cytoplasm, or plasma membrane of the tumor cells. Specific immunity to DNA virus-encoded nuclear antigens protects against tumor development in animals. For example, SV40-induced tumors in mice express antigens that induce specific protective immunity against subsequent challenge with other SV40-induced tumors, but not against tumors induced by other viruses. Because these antigens are targets for tumor transplant rejection, they are functionally defined as TSTAs, as are the TSTAs of chemically induced tumors described earlier. The viral TSTAs, however, are not unique for each tumor but are shared by all tumors induced by the same type of virus (Table 17-1).

Different effector mechanisms mediate rejection of DNA virus-induced tumors, and different viral antigens serve as the immunologic targets. For example, the T antigen is a virally encoded nuclear protein expressed in SV40 transformed cells. The T antigen is required to produce the malignant phenotype, and it is not part of infectious virus particles. Immunization of experimental animals with this protein induces protective immunity against the development of SV40-induced tumors, and this immunity is mediated by class I MHC-restricted CTLs. Human adenovirus-induced rodent tumors express a virally encoded protein called E1A, which is found largely in the nucleus and is the principal determinant of the transformed phenotype of the infected cells. E1A is not part of infectious adenovirus particles. When class I-restricted CTLs specific for a processed peptide derivative of the E1A protein are adoptively transferred into mice with adenovirus-induced tumors, these CTLs kill the tumors (Fig. 17-3). There is no comparably well characterized DNA virus-encoded tumor antigen that is known to induce protective immunity in human tumors.

Both humoral and cell-mediated immune responses to DNA virus-encoded proteins expressed on tumor cells are clearly demonstrable in animals and humans. A protective role of the immune system in controlling the growth of DNA virus-induced tumors is suggested by the higher frequency of these tumors in immunodeficient individuals. In humans, EBV-associated lymphomas and HPV-associated skin cancers arise much more frequently in immunosuppressed individuals, such as allograft recipients receiving immunosuppressive therapy and acquired immunodeficiency syndrome (AIDS) patients, than in normal individuals. Adenovirus infection induces tumors much more frequently in neonatal or nude (congenitally T cell-deficient) mice, compared with nor-

BOX 17–2. THE RELATIONSHIPS BETWEEN EPSTEIN-BARR VIRUS, MALIGNANCY, AND IMMUNODEFICIENCY



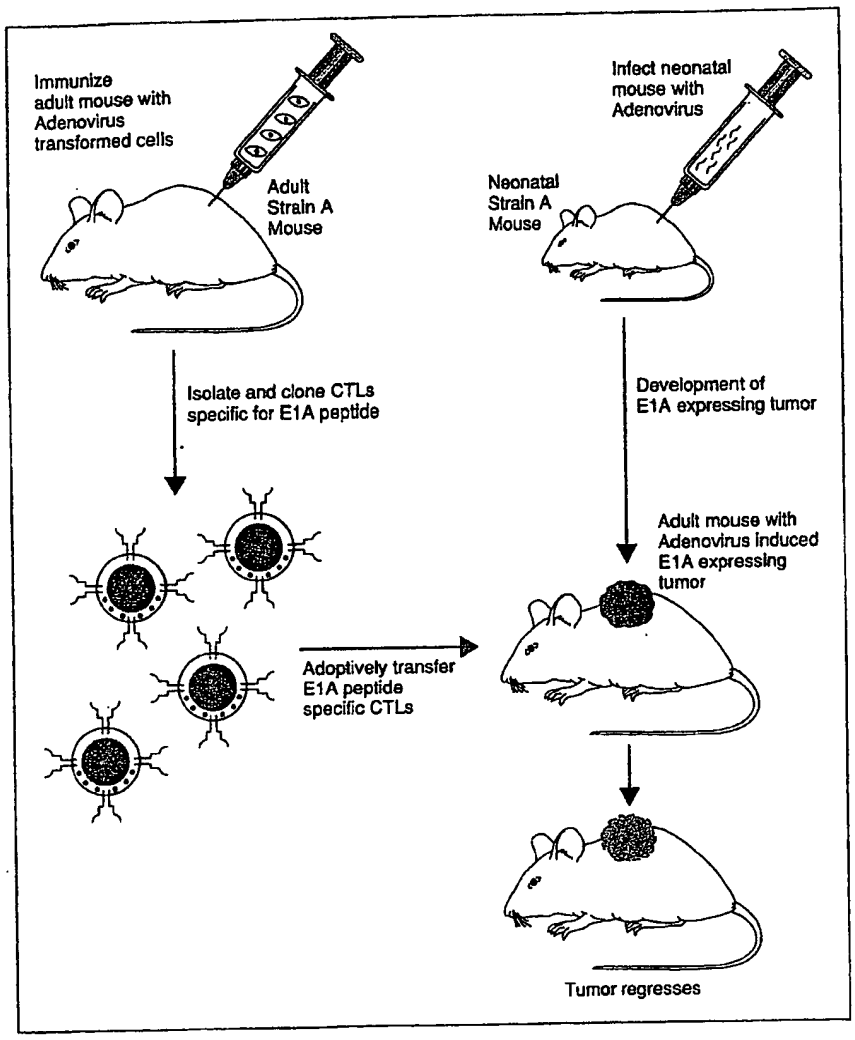


FIGURE 17–3. Viral antigen-specific cytolytic T lymphocytes (CTLs) kill virally infected tumors in vivo. *If neonatal mice are infected with adenovirus, they develop malignant tumors as adults and these tumors express the virally encoded E1A protein. The CTL clones isolated from a syngeneic mouse immunized with E1A-expressing cells can kill these E1A-expressing tumors when the CTLs are adoptively transferred to the tumor-bearing animal.*

mal adult mice. *Thus, a competent immune system may play a role in tumor immunosurveillance, not as a specialized anti-tumor function but because of its ability to recognize and kill virally infected cells.*

One of the clearest examples of viral oncogenesis is the development of tumors in animals infected with certain types of retroviruses (RNA tumor viruses). Some of these viruses carry well-defined oncogenes, induce tumors in days to weeks after infection, and are called acute transforming retroviruses. Examples of these acute transforming retroviruses include Rous sarcoma virus (carrying the *src* oncogene), avian myelocytomatosis virus (carrying the *myc* oncogene), and Kirsten murine sarcoma virus (carrying the v-K-*ras* oncogene). Other retroviruses, such as the murine leukemia viruses (MuLVs), cause tumors months after infection and do not carry any well-defined oncogenes. These slow-transforming retroviruses may cause tumors by inserting near, and deregulating transcription of, cellular genes that are responsible for growth control and differentiation.

The genomes of retroviruses are small, and there is a limited number of potentially immunogenic proteins that they may express in their host tumor cells. These proteins include products of the envelope *(env)* gene; core protein *(gag)* gene; and, in the case of acute transforming retroviruses, the oncogene. Retroviral oncogenes represent slightly altered forms of normal mammalian cellular genes, and therefore the viral oncogene products are usually not highly immunogenic. In contrast, humoral and cell-mediated immune responses to the *env* and *gag* products on tumor cells can be observed experimentally. Furthermore, *env* and *gag* products behave as TSTAs, stimulating CTL-mediated rejection of transplanted tumors. These TSTAs are shared by all tumors induced by the same type of retrovirus.

The only well-established human RNA tumor

virus is human T lymphotropic virus-1 (HTLV-1), which is the etiologic agent for adult T cell leukemia/lymphoma (ATL), an aggressive malignant tumor of $CD4^+$ T cells. Although immune responses specific for HTLV-1 encoded antigens have been demonstrated, it is not clear whether they play any role in protective immunity against development of tumors in virally infected people. Furthermore, ATL patients are often profoundly immunosuppressed, perhaps because of an effect of the virus on $CD4^+$ T cells, which the virus preferentially infects.

TISSUE-SPECIFIC (DIFFERENTIATION) ANTIGENS ON TUMOR CELLS

Tissue-specific, or differentiation, antigens are present on the surfaces of normal cells and are characteristic of a particular tissue type at a particular stage of normal differentiation of that tissue. Tumors that arise from a certain tissue often express the differentiation antigens of that tissue. Since these antigens are part of normal cells, they do not stimulate immune responses against the tumors on which they are expressed. The clinical significance of differentiation antigens on tumors relates to their use as targets for immunotherapy, discussed later, and also as diagnostic markers of the tissue of origin of tumors. The histologic appearance of a tumor may not be characteristic enough to permit a diagnosis of the type of normal tissue from which the tumor arose. Therefore, antibody probes for the expression of tissue-specific antigens may be required. For example, malignant lymphomas arising from the malignant transformation of a developing B cell may often be diagnosed as a B cell lineage tumor by the detection of a surface marker characteristic of normal pre-B cells, called CD10 (previously called common acute lymphocytic leukemia antigen, or CALLA). Tumors arising from more mature B cells are characterized by the presence of surface immunoglobulin. Examples of tissue-specific antigens expressed on tumors are listed in Table 17-2.

TABLE 17-2. Examples of Tissue-Specific Tumor Antigens Used in Clinicopathologic Analysis of Tumors

Tissue of Origin	Tumor	Antigens
B lymphocytes	B cell leukemias and lymphomas	CD10 (CALLA) Immunoglobulin
T lymphocytes	T cell leukemias and lymphomas	Interleukin-2 receptor (p55 chain) T cell receptor CD45R CD4/CD8
Prostate	Prostatic carcinoma	Prostate-specific antigen Prostatic acid-phosphatase
Neural crest-derived	Melanomas	S-100
Epithelial cells	Carcinomas	Cytokeratins

Abbreviations: CALLA, common acute lymphocytic leukemia antigen.

THE ROLE OF MHC MOLECULES IN ANTI-TUMOR IMMUNITY

The expression of MHC proteins on tumor cells may be critical for immunologic recognition and destruction of the tumor cells. This is clearly the case if T cells are required for the cognitive and/or effector stages of specific anti-tumor immune responses, since T cells can recognize antigens only in association with MHC molecules. It is possible, therefore, that tumors that stimulate protective immune responses express adequate amounts of MHC molecules whereas other tumors that are not immunogenic fail to express enough or any MHC molecules. However, when the level of MHC expression on a broad range of experimentally induced or human tumor cells is compared with the growth properties of those cells, no clear correlation exists. For example, metastatic tumors, which presumably have evaded immune attack, do not express, on the average, any more or less MHC proteins than non-metastatic tumors. Although extensive analysis of the role of MHC expression on tumor growth *in vivo* has not permitted us to make any broadly applicable conclusions, some experimental models have established the importance of the MHC in the immune response to certain virally induced tumors.

Resistance to induction of neoplasms by tumor viruses often correlates with MHC gene haplotypes in inbred animals. For example, some murine RNA tumor viruses induce tumors only in some inbred strains of mice and not others, suggesting a requirement that certain MHC alleles be expressed in order for an anti-tumor immune response to occur. Such an immune response may be largely specific for a particular viral protein. If the immunodominant peptide from that protein binds only to a particular class I MHC allele, that peptide will be immunogenic only in strains of mice that express the allele. Thus, only certain inbred strains of mice will mount a protective anti-tumor immune response against tumors expressing the viral antigen. This is an example of an immune response (Ir) gene effect linked to class I rather than class II MHC molecules.

A more direct experimental analysis of the effects of MHC expression on tumor growth *in vivo* has been performed using rat cells transformed *in vitro* with viral oncogenes and then transplanted into syngeneic immunocompetent rats. We have described previously that the adenovirus E1A gene product is a tumor antigen that serves as a target for class I-restricted CTLs. Rat cells transformed by the Ad12 strain of adenovirus readily grow into tumors when injected into animals. In contrast, Ad5 strain adenovirus-transformed cells are not tumorigenic. This difference is correlated with the profound suppression of class I MHC expression in cells transformed by

Ad12, but not Ad5, virus. The suppression of MHC expression is an effect of the adenovirus E1A oncogene, but neither the mechanism of suppression nor the molecular basis of the differences between Ad5 and Ad12 strains is well understood. When class I MHC expression on AD12-transformed cells is increased by γ-interferon (IFN-γ) treatment or by transfection of autonomously transcribed class I MHC genes, these cells acquire the same non-tumorigenic phenotype as their Ad5-transformed counterparts (Fig. 17-4). Thus, class I expression is apparently required for inhibition of tumor growth in this model, consistent with the finding that class I MHC-restricted CTLs mediate killing of adenovirus-induced tumors. However, differences in the *in vivo* growth of Ad5 versus Ad12 transformed cells may be influenced by other factors, besides class I MHC expression, such as resistance to lysis by NK cells.

EFFECTOR MECHANISMS IN ANTI-TUMOR IMMUNITY

Tumor antigens elicit both humoral and cell-mediated immune responses *in vivo*, and many immunologic effector mechanisms are capable of killing tumor cells *in vitro*. The challenge for tumor immunologists is to determine which, if any, of these effector mechanisms are important in protective immune responses

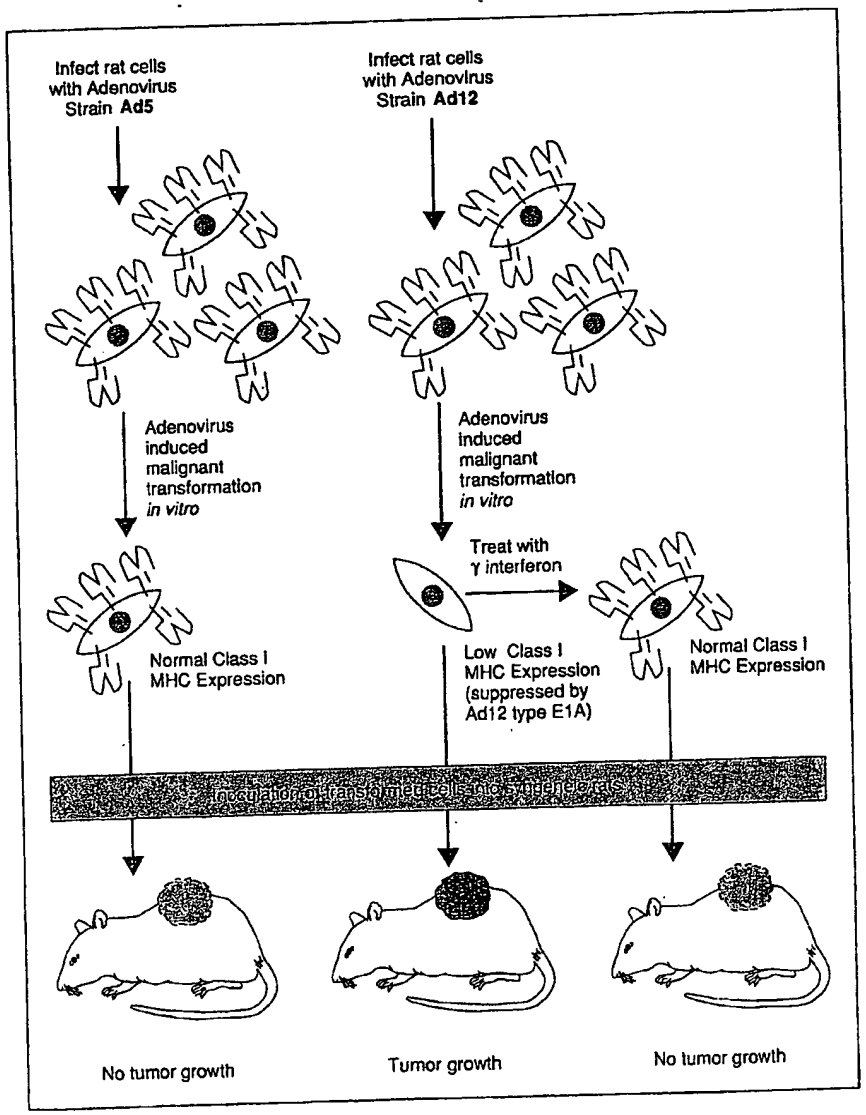


FIGURE 17-4. Relationship between class I MHC expression and tumorigenicity in adenovirus-induced tumors. *Rat cells that are malignantly transformed in vitro by infection with the Ad5 strain of adenovirus express normal levels of class I MHC molecules and are not tumorigenic in syngeneic rats. In contrast, rat cells that are malignantly transformed in vitro by infection with the Ad12 strain of adenovirus express low levels of class I MHC molecules and are tumorigenic. Ad12-infected tumors can be induced to express higher levels of class I MHC molecules by γ-interferon, and this treatment renders them non-tumorigenic. An interpretation of this experiment is that class I MHC expression on a virally induced tumor permits the host animal to mount a protective immune response, presumably against a virally encoded antigen presented by the tumor cell in association with class I MHC molecules.*

to spontaneously arising (non-experimental) tumors. In this section of the chapter, we briefly review the evidence for tumor killing by these various effector mechanisms and discuss which are the most likely to be relevant to human tumors.

Antibody Responses

Tumor-bearing hosts mount **antibody responses** specific for tumor antigens. The antigens that stimulate these immune responses are predictably limited to proteins that have not been expressed on normal tissues in a way that would induce tolerance. For example, as mentioned above, antibodies specific for the Tla are easily detected in normally Tla-negative mice bearing thymic leukemias. Patients with EBV-associated lymphomas have serum antibodies against EBV-encoded antigens expressed on the surface of their tumor cells. No evidence exists, however, for a role of such humoral responses in inhibiting tumor development or growth. A great variety of tumor cells can be lysed by antibody-dependent mechanisms *in vitro*. In these experimental situations, the antibodies against tumor surface proteins are often generated in other species and their tumoricidal activity is attributable to complement activation or to antibody-dependent cell-mediated cytotoxicity (ADCC) in which Fc receptor-bearing macrophages or NK cells mediate the killing. Whether or not these antibody-dependent mechanisms of tumor killing play a role *in vivo* remains unknown.

Cytolytic T Lymphocytes

CTLs provide effective anti-tumor immunity *in vivo*, as demonstrated in experimental tumor transplantation studies discussed earlier. In these cases, the effector cells are predominantly class I MHC-restricted CTLs which are fundamentally similar to virus specific or alloreactive CTLs described in Chapters 12 and 16. As discussed previously, the role for CTLs in immunosurveillance of non-virally induced tumors is questionable, since such tumors do not arise frequently in T cell-deficient animals or people or in patients with suppressed T cell immunity caused by therapeutic drugs or human immunodeficiency virus (HIV) infection. On the other hand, peripheral blood lymphocytes from patients with advanced tumors, including carcinomas and melanomas, contain CTLs that lyse explanted tumors from the same patients. Furthermore, mononuclear cells derived from the inflammatory infiltrate in human solid tumors, called tumor-infiltrating lymphocytes (TILs), also include CTLs with the capacity to lyse the tumor from which they were derived. However, the specificity of the anti-tumor CTLs derived from peripheral blood or tumors is not well established, since they often also show reactivity against unrelated tumor cells.

Natural Killer Cells

NK cells may be effector cells of natural and acquired immune responses to tumors. They utilize the same lytic mechanisms as CTLs to kill cells; however, they do not express T cell antigen receptors, and they kill targets in an MHC-unrestricted manner (see Chapter 12). NK cells lyse both virally infected cells and certain tumor cell lines, especially hematopoietic tumors, *in vitro*. In fact, lysis of such lines serves as a bioassay for NK activity. There appears to be a degree of specificity to NK killing, since many virally infected cells or tumor cells and most normal cells are not susceptible to NK lysis *in vitro*. The basis of this specificity is not understood. In addition, NK cells can be targeted to antibody-coated cells because they express low-affinity Fc receptors (CD16) for IgG molecules. The tumoricidal capacity of NK cells is increased by cytokines, including interferons, tumor necrosis factor (TNF), and interleukin-2 (IL-2). Therefore, their role in anti-tumor immunity may depend on the concurrent stimulation of T cells and macrophages which produce these cytokines. There is great interest in the role of IL-2 activated NK cells in tumor killing. These cells, called **lymphokine-activated killer (LAK) cells**, are derived *in vitro* by high-dose IL-2 treatment of peripheral blood cells or TILs from tumor patients. LAK cells exhibit a markedly enhanced and nonspecific capacity to lyse other cells, including tumor cells. The use of LAK cells in adoptive immunotherapy of tumors is discussed later.

A possible role for NK cells in tumor immunity *in vivo* is suggested by a variety of indirect evidence. For example, the incidence of tumors in different strains of inbred mice, or in mice of different ages, correlates inversely with the functional capacity of NK cells in these mice. Interestingly, T cell-deficient nude mice have normal or elevated numbers of NK cells and they do not have a high incidence of spontaneous tumors. Thus, it is possible that NK cells play a role in immunosurveillance against developing tumors, especially those expressing viral antigens. However, there is not a high degree of NK activity in the cellular infiltrates associated with solid human tumors, before *in vitro* expansion with IL-2.

Macrophages

Macrophages are potentially important cellular mediators of anti-tumor immunity. Their role is largely inferred from the demonstration that activated macrophages preferentially lyse tumor cells and not normal cells *in vitro*. The basis for this preferential susceptibility of tumor cells to macrophage mediated lysis is unknown. Like NK cells, macrophages express Fcγ receptors and they can be targeted to tumor cells coated with antibody. There are probably several mechanisms of macrophage killing of tumor target cells, some of which are essentially the same as the mechanisms of macrophage killing of infectious orga-

nisms. These include the release of lysosomal enzymes and reactive oxygen metabolites. Other reactive chemical species, such as nitric oxide, may also play a role.

Activated macrophages also secrete the cytokine TNF, which, as its name implies, was first characterized as an agent which can kill tumors but not normal cells. The various actions of TNF have been discussed in Chapter 11. There is convincing evidence that a major component of macrophage-mediated killing of tumors is due to TNF secretion. For example, tumor cells selected *in vitro* for resistance to killing by TNF are often also resistant to killing by macrophages. Killing by both mechanisms is slow (24 to 48 hours), is augmented by protein or RNA synthesis inhibitors, and involves nuclear DNA fragmentation rather than osmotic lysis.

TNF kills tumors by at least two different mechanisms. First, *binding of TNF to high-affinity cell surface receptors is directly toxic to tumor cells.* The toxicity may be a result of the production of free radicals. Normal cells respond to TNF by synthesizing superoxide dismutase, an enzyme that participates in the inactivation of free radicals. In contrast, many tumor cells fail to make superoxide dismutase in response to TNF. Thus, part of the explanation of selective tumor cell killing by TNF may be loss of responses in these cells that serve to protect normal cells. Direct toxic effects of TNF may also involve disruption of cytoskeletal proteins, or interference with gap junction formation. Second, *in vivo, TNF causes tumor necrosis by mobilizing various host responses.* In fact, even tumor cells lacking TNF receptors can be eradicated in mice by treatment with TNF. The key observation is that TNF selectively eradicates vascularized tumors and is much less effective in killing avascular implants. Histologically, the response to TNF, described as hemorrhagic necrosis, looks very much like the localized Shwartzman reaction described in Chapter 11. This resemblance has led to the suggestion that TNF acts selectively on tumor vessels to produce a Shwartzman-like reaction leading to thrombosis of the vessels and ischemic necrosis of tumors. Tumor vessels, unlike normal vessels, may be already "primed" to trigger the Shwartzman response once they encounter TNF. The differences between normal and tumor vessels are a subject of intense study.

MECHANISMS OF EVASION OF THE IMMUNE SYSTEM BY TUMORS

Although many malignant tumors are weakly immunogenic, there are numerous examples of tumor antigens that can stimulate strong immune responses. A major focus of tumor immunology is to understand the ways in which tumor cells evade immune destruction, despite their potential immunogenicity. The process of evasion, often called "tumor escape," may be a result of one or more mechanisms.

1. Some tumors may be poorly immunogenic in a particular host because the host does not express the appropriate MHC molecules necessary for binding and presenting processed derivatives of tumor antigens. This immune response gene effect is hypothesized to be the reason why some strains of mice are resistant to tumor induction by murine leukemia viruses whereas others are not.

2. MHC expression may be down-regulated on tumor cells so that they cannot form immunologically recognizable complexes of processed tumor antigens and MHC molecules. We have previously discussed the correlation of MHC down-regulation and tumorigenicity in adenovirus-induced tumors.

3. A host may be tolerant to some tumor antigens, either because of neonatal exposure to such antigens or because the tumor cell may present its antigens to the immune system in a tolerogenic form, e.g., in high doses or without the proper costimulators (see Chapter 10). Neonatally induced tolerance has been demonstrated for tumors caused by the murine mammary tumor virus. This virus causes breast tumors in adult mice that have acquired the viral infection by neonatal nursing. Although these tumors are not seen as foreign in these mice and do not stimulate an immune response, they are highly immunogenic when transplanted to syngeneic virus-free mice. Another example of neonatally induced tolerance to virally encoded tumor antigens is seen in SV40-transgenic mice. Strains of SV40-transgenic mice that express SV40 genes during early development and have a high incidence of tumors do not mount immune responses against the SV40 T antigen. In contrast, other SV40-transgenic mice that have a low incidence of tumors are immunologically reactive to the SV40 T antigen.

4. The kinetics of tumor growth may allow for the establishment of immunologically resistant tumors before an effective immune response develops. This phenomenon, called "sneaking through," has been experimentally modeled by transplantation studies. Transplantation of small numbers of tumor cells may lead to the establishment of lethal tumors, whereas larger transplants of the same tumor are rejected. One postulated reason for this apparent contradiction is that low doses of tumor antigens are not sufficiently stimulatory to the immune system, and by the time a large number of tumor cells grow in the transplant recipient, mutations in tumor antigen genes may have occurred that reduce the chance of immune recognition.

5. Anti-tumor immunity may result in selection of mutant tumor cells that have lost expression of immunogenic proteins, especially if such proteins are not critical for the malignant phenotype of the tumor. Given the generally high mitotic rate of tumor cells and their genetic instability, such mutations are theoretically likely. Analysis of tumors that are serially transplanted from one animal to another has shown that the loss of antigens recognized by tumor-specific CTL clones correlates with increased growth and metastatic potential.

6. The loss of surface expression of tumor antigens as a result of antibody binding, called **antigenic modulation**, leads to acquired resistance to immune effector mechanisms. Antigenic modulation is due to either endocytosis or shedding of the antigen-antibody complexes. Thus, some non-complement-fixing anti-tumor antibodies may protect tumor cells from other, complement-activating antibodies. Antigenic modulation is perhaps most relevant as a problem complicating attempted passive immunotherapy with anti-tumor antibodies.

7. Antigens shed by tumors, and complexes of antibody with shed tumor antigens, have been postulated in the past to act as blocking factors that interfere with immune responses to tumors. The mechanisms of action of blocking factors remain obscure but could involve functional blockade of NK cell Fc receptors, or induction of "suppressor cells" which specifically down-regulate the function of tumor antigen-specific helper T cells.

8. Tumor cell surface antigens may be hidden from the immune system by glycocalyx molecules, including sialic acid-containing mucopolysaccharides. This is called "antigen masking" and may be a consequence of the fact that tumor cells often express more of these glycocalyx molecules than do normal cells. Similarly, some tumors may shield themselves from the immune system by activating the coagulation system, thereby investing themselves in a "fibrin cocoon."

9. Immunosuppression may be induced by tumor products or by the chemical, physical, or infectious agents that induce malignant transformation of cells. An example of an immunosuppressive tumor product is transforming growth factor-β (TGF-β), which is secreted in large quantities by many tumors. TGF-β inhibits a wide variety of lymphocyte and macrophage functions (see Chapter 11). Immunosuppressive carcinogenic agents include ionizing radiation, chemotherapeutic agents, and certain viruses. These agents can kill or functionally inhibit lymphocytes.

IMMUNOTHERAPY OF TUMORS

The potential for treating cancer patients by immunologic approaches has held great promise for immunologists and cancer biologists over much of this century. Recent advances in our understanding of the immune system have encouraged a variety of new strategies. Next we describe some of the modes of tumor immunotherapy that have been tried in the past or are currently being investigated.

Stimulation of Immune Effectors

The development of virally induced tumors can be blocked by vaccination with viral antigens. This approach is successful in reducing the incidence of feline leukemia virus-induced hematologic malignancies in cats and in preventing the herpesvirus-induced lymphoma called Marek's disease in chickens. In humans, it is possible that the ongoing vaccination program against the hepatitis B virus may reduce the incidence of hepatocellular carcinoma, a cancer that is associated with HBV infection of the liver.

Many different approaches have been used for the immunotherapy of already established tumors. Nonspecific immune stimulation of tumor patients with adjuvants, such as the bacille Calmette-Guérin (BCG) mycobacterium injected at the sites of tumor growth, has been tried many times. This treatment serves predominantly to activate macrophages. Oncologists are still assessing the potential of local BCG administration in bladder carcinomas and melanomas. Another experimental approach to nonspecific immune stimulation is the administration of low doses of anti-CD3 antibodies to mice with transplanted fibrosarcomas. This treatment results in polyclonal activation of T cells and, concomitantly, prevention of tumor growth. The dose of anti-CD3 is of critical importance since, as described in Chapter 16, high doses of anti-CD3 antibody are widely used as an immunosuppressant to prevent allograft rejection.

Immunization of tumor-bearing hosts with tumor cells is an experimental approach previously attempted in experimental animals and in humans. Leukemic patients have been immunized with killed leukemic cells from other patients with little success. In attempts to make them more immunogenic, animal tumors have been altered by covalently linking haptens (such as trinitrophenol) to their surface or by infecting the tumor cells with viruses (such as vaccinia virus). These altered cells are then used to immunize tumor-bearing animals. The rationale for this approach is based on the assumption that immune responses to the altered tumor cells will then be effective on the unaltered tumor cells, although the basis for this cross-reaction is not clear. The results suggest that such procedures may enhance active anti-tumor immunity, but their feasibility in the clinical situation is unproven.

Antibody Therapies

There are many variations on the use of *antibodies specific for tumor antigens* in tumor therapy (Table 17-3). The theoretical potential of using tumor-specific antibodies as "magic bullets" remains alluring to many investigators:

1. *Anti-idiotypic antibodies* have been used in the treatment of B cell lymphomas expressing surface Ig with particular idiotypes. The idiotype is a highly specific tumor antigen, since it is expressed only on the neoplastic clone of B cells and on no other cells. (Anti-idiotypic antibodies are raised by immunizing rabbits with a patient's B cell tumor, and depleting the serum of reactivity against all other human immunoglobulins.) This strategy relies on complement fixation or ADCC in order for the lymphoma cells to be killed. The approach is not generally successful, and

TABLE 17-3. Examples of Immunotherapy with Anti-tumor Antibodies

Approach	Examples	Tumors	Current Status
Free antibody	Anti-Ig idiotype	B cell lymphomas	Human trials
	Anti-IL-2R	T cell lymphomas	Human trials
	Anti-ganglioside	Melanoma	Human trials
	Anti-*neu* oncogene product	Sarcoma	*In vivo* animal model
Ig-toxin conjugates	Ricin A-anti-CD5	T cell lymphomas	*In vitro*
	Ricin A-anti-CD22	B cell lymphomas	Human trials
	Ricin A-anti-CD19	B cell lymphomas	*In vitro*
	Ricin A-anti-melanoma	Melanoma	Human trials
Ig-drug conjugates	Chlorambucil-anti-melanoma	Melanoma	Human trials
Ig-radioisotope conjugates	^{211}Bismuth-anti-Thy-1	T cells	*In vitro*
Dual-specificity heteroconjugate Ig	Anti-CD3:Anti-TAA	Sarcoma	*In vitro*
Ig-hormone heteroconjugate	Anti-CD3: Melanocyte-stimulating hormone	Melanoma	*In vitro*

Abbreviations: Ig, immunoglobulin; IL-2R, interleukin-2 receptor; TAA, tumor-associated antigen.

there are many theoretical reasons why it may not work. Since surface Ig expression is not functionally related to the malignant phenotype of the cell, selective outgrowth of non-Ig-expressing tumor cells can occur. Alternatively, the high degree of somatic mutation known to occur in Ig genes could result in the selective outgrowth of tumor cells with altered idiotypes no longer reactive with the anti-idiotypic antibody. Furthermore, since the rabbit antibodies are foreign proteins, the tumor patient may develop anti-rabbit Ig antibodies and these may interfere with the efficacy of the rabbit-anti-tumor antibodies. Attempts to circumvent these problems by producing human monoclonal antibodies or by injecting cocktails of several different antibodies have also not proven successful.

2. *Antibodies directed against growth factor receptors (IL-2 receptors)* have been used in the experimental therapy of human T lymphocyte malignancies, including HTLV-1 associated leukemias and lymphomas. The rationale of this approach is that IL-2 may serve to stimulate the growth of these tumor cells, and such antibodies may cause modulation or functional blockade of IL-2 receptors (IL-2R). Alternatively, such antibodies could cause complement-mediated lysis of the IL-2R expressing tumor cells. Anti-IL-2R therapy is not tumor specific and may be immunosuppressive because normal T cells would be rendered nonfunctional. There is also no evidence to support an obligatory role of IL-2 as an autocrine or paracrine growth factor for HTLV-1-induced tumors *in vivo*. Although initial trials have met with little success, anti-growth factor receptor antibodies could theoretically be useful for the treatment of other tumors.

3. *Antibodies specific for an oncogene product* might be able to inhibit tumor growth if that oncogene product is essential for the transformed phenotype. Monoclonal antibodies against the *neu* oncogene encoded cell surface protein cause *neu*-transformed cells to revert to a nontransformed phenotype *in vitro*, and the same antibodies can inhibit tumor growth in mice.

4. *Anti-tumor antibodies coupled to toxic molecules, radioisotopes, and drugs* are being used in immunotherapy trials in cancer patients and in experimental animals. Toxins such as ricin or diphtheria toxin are highly potent inhibitors of protein synthesis and are theoretically useful at extremely low doses if they are bound to tumor-specific antibodies to form **immunotoxins.** This approach requires the covalent attachment of the toxin to an antibody molecule without loss of toxicity or antibody specificity. Furthermore, the immunotoxin must be endocytosed and delivered to the appropriate intracellular site of action. Another approach is to covalently attach anti-neoplastic drugs or cytocidal radioisotopes to anti-tumor antibodies. Two practical difficulties must be overcome for this technique to be successful. First, the specificity of the antibody must be such that there is not significant binding to non-tumor cells. As we have discussed, there are few truly tumor-specific antigens to select when designing an antibody-based immunotherapy approach. Second, it may be difficult to ensure that a sufficient amount of antibody reaches the appropriate target, before clearance of the antibody by Fc receptor-bearing phagocytic cells. Such clearance may not only reduce anti-tumor effectiveness, but also may damage phagocytic cells. $F(ab')_2$ conjugated toxins may minimize the latter problem.

5. *Heteroconjugate antibodies* may allow targeting of cytotoxic effector cells onto tumor cells. In this approach, an antibody specific for a tumor antigen is covalently coupled to an antibody directed against a surface protein on cytotoxic effector cells, such as NK cells or CTLs. Such heteroconjugates promote binding of the NK cells or CTLs to appropriate tumor targets. A heteroconjugate consisting of an anti-CD3 antibody coupled to an antibody against a tumor cell surface protein enhances CTL-mediated lysis of the tumor

cell. In this case, the anti-CD3 antibody serves not only to bring the CTL into proximity of the target cell but also to activate the CTL.

6. *Conjugates of antibodies and hormones* can be used to target CTLs to tumor cells expressing hormone receptors. For example, anti-CD3 antibodies coupled to melanocyte-stimulating hormone enhance *in vitro* destruction of hormone-binding human melanoma cells by CTLs. A related (but not strictly immunologic) strategy is to generate fusion proteins with toxic activity and tumor-binding capacity. This is done by engineering and expressing genetic constructs in which bacterial toxin genes are linked to the genes encoding the binding domains of hormones or other ligands that bind to the tumor cells. Fusion proteins combining IL-2 and protein toxins have been used as T cell lytic agents in experimental transplantation (see Chapter 16).

7. *In vitro depletion of bone marrow tumor cells by antibody plus complement-mediated lysis* is used in autologous bone marrow transplants in B cell lymphoma patients. In this protocol, some of the patient's bone marrow is removed and the patient is given lethal doses of irradiation and chemotherapy, which destroy tumor cells. This treatment also destroys the remaining normal marrow cells in the patient. The bone marrow that was removed earlier is then treated with antibodies directed against B lymphocyte-specific antigens, which are expressed on the B cell-derived lymphoma cells. Complement is then added to promote lysis of the lymphoma cells that have bound antibody. The bone marrow, having been purged of lymphoma cells, is reinjected into the patient to reconstitute the hematopoietic system destroyed by irradiation and chemotherapy.

Adoptive Cellular Immunotherapy

Adoptive cellular immunotherapy refers to the transfer of cultured immune cells that have anti-tumor reactivity into a tumor-bearing host. Two variations to this approach are currently in clinical trials:

1. *Lymphokine-activated killer cell therapy* involves the *in vitro* generation of LAK cells by culturing peripheral blood leukocytes removed from tumor patients in high concentrations of IL-2. The LAK cells are then injected back into the cancer patient. As we discussed previously, LAK cells are derived mainly from NK cells. Adoptive therapy with autologous LAK cells, in conjunction with *in vivo* administration of IL-2 or chemotherapeutic drugs, has had impressive results in mice, with regression of solid tumors. Human LAK therapy trials have so far been largely restricted to advanced cases of metastatic tumors, and the efficacy of this approach cannot yet be fully evaluated.

2. *Tumor-infiltrating lymphocyte therapy* involves the generation of LAK cells from mononuclear cells originally derived from the inflammatory infiltrate present in and around solid tumors, obtained from surgical resection specimens. The rationale for this approach is that TILs may be enriched for tumor-specific killer cells. In fact, TILs include activated NK cells and CTLs, both of which appear to kill cells nonspecifically. High doses of IL-2 may impart CTLs with the capacity to kill targets without specific T cell receptor (TCR)-mediated binding. Human trials with TIL therapy are ongoing.

Cytokine Therapy

Cytokines are also used for the treatment of various tumors. This type of experimental therapy has become feasible only recently with the production of highly purified or recombinant cytokines in sufficient quantities. The rationale for using cytokines is based on their ability to enhance one or more components of cellular immune function; the effects of the cytokines are not specific for anti-tumor-directed immune effector cells.

1. IL-2, administered in high doses, is being used alone or in conjunction with adoptive cellular immunotherapy. This treatment is effective in inducing measurable tumor regression in 20 to 40 per cent of patients with melanoma and renal cell carcinoma. Presumably, the IL-2 works by activating NK cells and/or CTLs, i.e., inducing LAK cell differentiation *in vivo*. The treatment can be highly toxic, causing fever, pulmonary edema, and often shock. These toxic effects are probably indirectly mediated by IL-2 acting on other lymphocytes to enhance production of TNF, IFN-γ, and lymphotoxin. Interleukin-4 (IL-4) also can activate CTLs and is currently being tested in clinical trials as an alternative agent with potentially fewer side effects.

2. TNF has been used in preliminary cancer treatment protocols in patients with advanced carcinomas. Although TNF clearly has potent anti-tumor effects *in vitro*, it has many undesirable pathologic effects and can be highly toxic at the doses that are required for tumor killing *in vivo*.

3. Alpha-interferon (IFN-α) is a type I interferon, produced largely by leukocytes (see Chapter 11). It has antiproliferative effects on cells *in vitro*, increases the lytic potential of NK cells, and increases class I MHC expression on various cell types. This cytokine has been used in extensive clinical trials, with promising results. Objective tumor regression responses occur in 10 to 15 per cent of renal cell carcinomas, melanomas, and Kaposi sarcomas; 40 to 50 per cent of various lymphomas; and 80 to 90 per cent of hairy cell leukemias (a B cell lineage tumor). In fact, IFN-α treatment of hairy cell leukemia is now standard practice and is currently the only reliable cytokine therapy for a human cancer.

4. IFN-γ has been used in clinical trials for the treatment of various hematopoietic and solid tumors, with little success. It was hoped that the macrophage and NK cell-activating properties of this cytokine, as

well as its ability to up-regulate MHC molecule expression, would help to enhance anti-tumor immunity. Intraperitoneal administration of IFN-γ for the treatment of ovarian carcinomas is currently being evaluated.

5. Hematopoietic growth factors, including granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are used in cancer treatment protocols, although not strictly to enhance immune responses against tumors. Rather, they shorten periods of neutropenia following chemotherapy or after autologous bone marrow transplantation by stimulating the maturation of granulocyte precursors.

An interesting experimental approach to cytokine treatment of tumors is the transfection of tumor cells *in vitro* with cytokine genes followed by transplantation of the cells into tumor-bearing animals. In this way, immunostimulatory cytokines are produced in abundance specifically at the site of tumor growth. This has been accomplished in different animal tumors with IL-2, IL-4, and IFN-γ genes. In each case, transfection of the cytokine gene inhibits tumor growth *in vivo* and in each case the inhibition is due to stimulation of a different immune effector mechanism by the secreted cytokine. For example, IL-4 transfected tumor cells stimulate an intense eosinophilic inflammatory response *in vivo* and do not grow into lethal tumors and IL-2 transfected colon carcinoma cells stimulate a protective CTL response in mice. The potential of this type of strategy for treatment of human tumors remains hypothetical.

SUMMARY

Malignant tumors express a variety of antigens that may stimulate and serve as targets for anti-tumor immunity. Protective anti-tumor immune responses have been convincingly demonstrated in experimental animal models. It has been more difficult to demonstrate that natural or acquired immune responses to most common human tumors serve to control their development or growth, but this may reflect the limitations of analysis of immune responses in humans. The development of tumors induced by viruses, which express virally encoded antigens, is likely to be inhibited by specific immune responses. Antigens unique to individual tumors, which stimulate specific rejection responses upon transplantation, have been demonstrated only in experimental animal tumors. Other tumor antigens that can stimulate immune responses are shared by different tumors, and these include viral antigens and products of derepressed genes. Tumors may also express tissue differentiation antigens or embryonic antigens to which the host is tolerant; these molecules are useful diagnostic markers. MHC molecule expression may vary from tumor to tumor; in some tumors, MHC expression may be necessary for protective immune responses.

Virtually every immunologic effector mechanism known can destroy tumor cells *in vitro*. One or more of these mechanisms may work on tumor cells *in vivo*, and different mechanisms may be effective on different tumors. Natural killer cells, CTLs and macrophages are probably the major effectors of anti-tumor immunity *in vivo*. Various mechanisms have been proposed to explain how potentially immunogenic tumors escape destruction by the immune system. These mechanisms include MHC-linked genetic unresponsiveness of the host, down-regulation of MHC molecules, induction of tolerance to tumor antigens, loss of expression of immunogenic proteins due to mutations, modulation of tumor antigens by anti-tumor antibodies, antigen masking by extracellular proteins, and immunosuppression of the host. A variety of immunologic approaches for treating cancers, including anti-tumor antibodies, adoptive cellular immunotherapy, and cytokine treatment are currently in clinical trials.

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Exhibit 2-4

CHAPTER EIGHTEEN

DISEASES CAUSED BY HUMORAL AND CELL-MEDIATED IMMUNE REACTIONS

Specific immunity is a powerful homeostatic mechanism for eliminating pathogenic microbes and other foreign antigenic substances. The effector mechanisms of specific immunity, such as complement, phagocytes, inflammatory cells, and cytokines, are not themselves specific for foreign antigens. Therefore, immune responses and attendant inflammation are often accompanied by local and systemic injury to normal self tissues. Normally, however, such pathologic side effects are controlled and self-limited and they abate as the foreign antigen is eliminated. Furthermore, normal individuals are tolerant of their own antigens and do not develop immune responses against autologous tissues. *Failure to control physiologic immune responses against foreign antigens or to maintain self-tolerance leads to diseases in which the primary pathogenic mechanism is immunologic.* Disorders that result from aberrant, excessive, or uncontrolled immune reactions are also called **hypersensitivity diseases.** This term arises from the clinical definition of immunity as "sensitivity," which is based on the observation that an individual who is immune to an antigen responds to, or is "sensitive to," exposure to that antigen. (As applied to the historical definition of immunity to microbes, such a "sensitive" individual, of course, would usually be resistant to infection by that microbe.) Immunologic diseases that are thought to be due to immune responses against self antigens are called **autoimmune diseases.**

In this chapter we first discuss the mechanisms by which humoral and cell-mediated immune responses lead to diseases, using examples of clinical and experimental disorders to illustrate the current understanding of their etiology and pathogenesis. We then discuss the mechanisms that might lead to autoimmunity and describe some of the approaches that are being used to elucidate these mechanisms.

TYPES OF IMMUNOLOGIC DISEASES

Immunologic diseases comprise a clinically heterogeneous group of disorders. The two principal factors that determine the clinical and pathologic manifestations of such diseases are (1) the type of immune response that leads to tissue injury, and (2) the nature and location of the antigen that initiates or is the target of this response.

The most frequently used *classification of immunologic diseases is based on the principal pathogenic mechanism responsible for cell and tissue injury* (Table 18-1). Immediate hypersensitivity caused by IgE antibodies and mast cells, which is also called type I hypersensitivity, has been described in Chapter 14. Antibodies other than IgE can cause tissue injury by recruiting and activating inflammatory cells and the complement system. These antibodies may be specifically reactive with one's own antigens or with foreign antigens that are deposited in or are antigenically cross-reactive with self antigens. Such disease-producing antibodies may be detectable in two forms. Some can be found bound to their target antigens or in the circulation in a free form, and the diseases they cause are called type II hypersensitivity. Other antibodies may form immune complexes in the circulation, and the complexes subsequently deposit in tissues, particularly in blood vessels, and cause injury. Diseases caused by immune complexes are classified under type III hypersensitivity. Finally, tissue injury may be due to activated T lymphocytes and the principal effector cells of delayed type hypersensitivity (DTH), namely activated macrophages; these are called type IV hypersensitivity disorders.

In our discussion, we will use descriptions that identify the pathogenic mechanisms rather than the less informative numerical designations. This classification is useful because distinct types of pathogenic immune responses show quite different patterns of tissue reactions and may vary in their tissue specificity. As a result, they produce disorders with distinct clinical and pathologic features. However, immunologic diseases in the clinical situation are often complex and are due to various combinations of humoral and cell-mediated immune responses and multiple effector mechanisms. This is not surprising, given that a

TABLE 18-1. Classification of Immunologic Diseases

Type of Hypersensitivity	Pathologic Immune Mechanisms	Mechanisms of Tissue Injury and Disease
Type I: Immediate hypersensitivity	IgE antibody	Mast cells and their mediators (vasoactive amines, arachidonic acid metabolites, cytokines)
Type II: Antibody-mediated	IgM, IgG antibodies against tissue or cell surface antigen	1. Complement activation 2. Recruitment and activation of leukocytes (neutrophils, macrophages) 3. Abnormalities in receptor functions
Type III: Immune complex-mediated	Immune complexes of circulating antigens and IgM or IgG antibodies	1. Complement activation 2. Recruitment and activation of leukocytes
Type IV: T cell-mediated	1. $CD4^+$ T cells (delayed type hypersensitivity) 2. $CD8^+$ CTLs (T cell-mediated cytolysis)	1. Activated macrophages, cytokines 2. Direct target cell lysis, ? cytokines

Abbreviations: Ig, immunoglobulin; CTL, cytolytic T lymphocyte.

single antigen may normally induce both humoral and cell-mediated immunity.

Immunologic diseases can also be subdivided based on the source of the antigens against which the pathogenic immune responses are directed. Such a classification is often impractical in the clinical situation, because in many immunologic disorders the antigen against which the pathologic immune response is generated has not been identified. Nevertheless, it is important to try to classify these diseases by the specificity of the immune response, because specificity may provide valuable insights into the mechanisms by which immunologic diseases are initiated.

1. *Immune responses to foreign antigens may be pathogenic in several situations.* First, some microbes persist for prolonged periods because they resist elimination by immune and inflammatory mechanisms. This leads to persistent antigenic stimulation, resulting in a response of increasing magnitude associated with severe tissue injury. Second, some foreign antigens may share antigenic determinants with self tissues and lead to immune responses that cross-react with self antigens. Third, the foreign antigen may be deposited or "planted" in a particular tissue because of a physicochemical affinity with normal tissue components, so that an immune response directed against the foreign antigen becomes targeted to the tissue in which this antigen is fixed. Fourth, normal immune responses may become defective in their self-regulation, so that they continue unabated even after the initiating foreign antigen is eliminated. Examples of diseases caused by these different kinds of immune responses to foreign antigens are mentioned later in this chapter.

2. *Immune responses against self (autologous) antigens, called **autoimmunity**, are usually abnormal.* In normal individuals, potentially self-reactive lymphocytes that encounter self antigens prior to attaining a stage of functional maturity are either deleted or inactivated (see Chapters 8 and 10). Many mechanisms have been implicated in the loss of self-tolerance and the induction of autoimmunity, and these are discussed later in this chapter. Pathologic autoimmunity is a frequent cause of immunologic diseases in humans, estimated to affect 1 to 2 per cent of the United States population.

Diseases Caused by Antibodies

The first immunologic diseases in which the pathogenic mechanisms were identified were diseases caused by deposition of antibodies in tissues. This was largely because techniques for detecting abnormal circulating autoantibodies and immunoglobulins (Ig) deposited in tissues were developed well before methods for identifying and isolating T cells from lesions or from the blood of patients. Moreover, in experimental models of immunologic diseases, it was possible to cause tissue injury by transferring purified Ig before pure or clonal populations of tissue-reactive T cells became available. For historic reasons, therefore, many of the general principles of immunologic diseases are based on antibody-mediated disorders.

Antibody-mediated diseases are of two types, which differ in their clinicopathologic manifestations and are due to the deposition of antibodies in distinct forms (Fig. 18-1):

1. *Immunologic diseases may be produced by **immune complexes** composed of a soluble antigen and specific antibody; such complexes are formed in the circulation and may deposit in vessel walls virtually anywhere in the body.* This leads to local activation of leukocytes and the complement system, with resultant tissue injury. The antigens that induce the pathogenic humoral immune response can be foreign or self antigens, and the antibodies in the complexes are usually IgM or IgG because these isotypes are most efficient at activating complement and/or inflammatory cells. The pathologic features of such diseases reflect the site(s) of immune complex deposition and are not determined by the cellular source of the antigen. Therefore, immune complex–mediated diseases tend to be systemic, with little or no specificity for a particular antigen located in a particular tissue or organ.

2. *Antibodies against circulating cells or fixed tissue antigens cause diseases that are specific for that cell or tissue.* The lesions are due to the binding of specific antibodies and not to the deposition of immune complexes formed in the circulation. In most cases, such antibodies are autoantibodies, although occasionally they may be produced against a foreign antigen that is immunologically cross-reactive with a component of self tissues. Such antibodies are usually of the IgM or IgG class, and they cause disease by activating the same effector mechanisms as immune complexes. Some immunologic diseases are due to antibodies specific for cellular structures, such as hormone receptors, that are important for normal function. In these situations, diseases may occur because of interference with the normal functions of these structures and not because of antibody-mediated inflammation or complement activation leading to actual tissue injury.

In order to prove that a particular disease is caused by antibodies, one would need to demonstrate that the lesions can be induced in a normal animal by the adoptive transfer of Ig purified from the blood or affected tissues of individuals with the disease. An experiment of nature is occasionally seen in children of mothers suffering from antibody-mediated diseases. These infants may be born with transient expression of the diseases because of transplacental passage of antibodies. However, in the usual clinical situations it is not possible to experimentally transfer diseases with antibodies. Therefore, the *diagnosis* of antibody-mediated disease is usually based on the following criteria: (1) the demonstration of antibodies or immune complexes deposited in tissues, (2) the presence of anti-tissue antibodies or immune complexes in the circulation, and (3) clinicopathologic similarities with experimental diseases that are proved to be antibody-mediated by adoptive transfer.

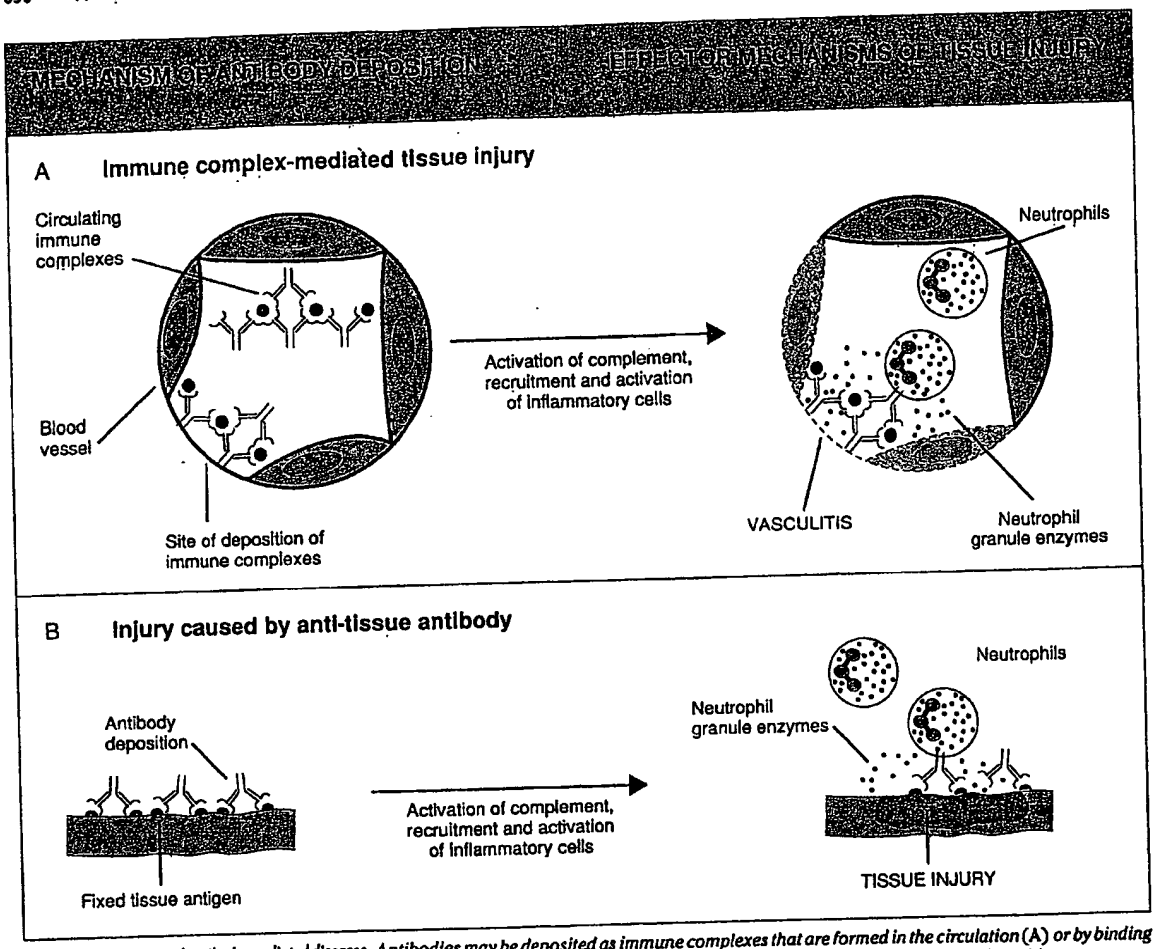


FIGURE 18–1. Types of antibody-mediated diseases. *Antibodies may be deposited as immune complexes that are formed in the circulation* (A) *or by binding specifically to tissue antigens* (B). *In both cases, similar effector mechanisms lead to tissue injury at the sites of antibody deposition.*

Mechanisms of Antibody-Mediated Tissue Injury and Functional Abnormalities

In normal immune responses, the protective functions of antibodies are mediated by neutralization of the antigen, activation of the complement system, and recruitment of host inflammatory cells. The same effector mechanisms are responsible for the pathologic consequences of antibody deposition. Which effector systems are involved in mediating the protective functions or pathologic effects of different antibodies is determined largely by the isotype of the Ig and the nature of the target antigen:

1. *Complement-mediated lysis of cells* occurs after IgM and some classes of IgG antibodies bind to their specific antigens (see Chapter 13). Complement activation leads to the generation of the membrane attack complex (MAC), which causes osmotic lysis of cells (Fig. 18–2*A*).

2. *Recruitment and activation of inflammatory cells,* mostly neutrophils and, to a lesser extent, monocytes, occur at sites of antibody deposition. This is largely in response to the local generation of complement by-products, particularly C5a (Fig. 18–2*B*). In addition, neutrophils and macrophages express surface receptors specific for the Fc portions of γ heavy chains and can, therefore, bind to and be activated by antigen-complexed IgG antibodies even in the absence of complement activation. Activated neutrophils and macrophages produce hydrolytic enzymes, reactive oxygen species, arachidonic acid metabolites, and cytokines, which can all contribute to cell and tissue injury.

3. *Phagocytosis of antibody-coated cells* (Fig. 18–2*C*) may lead to selective depletion of those cells.

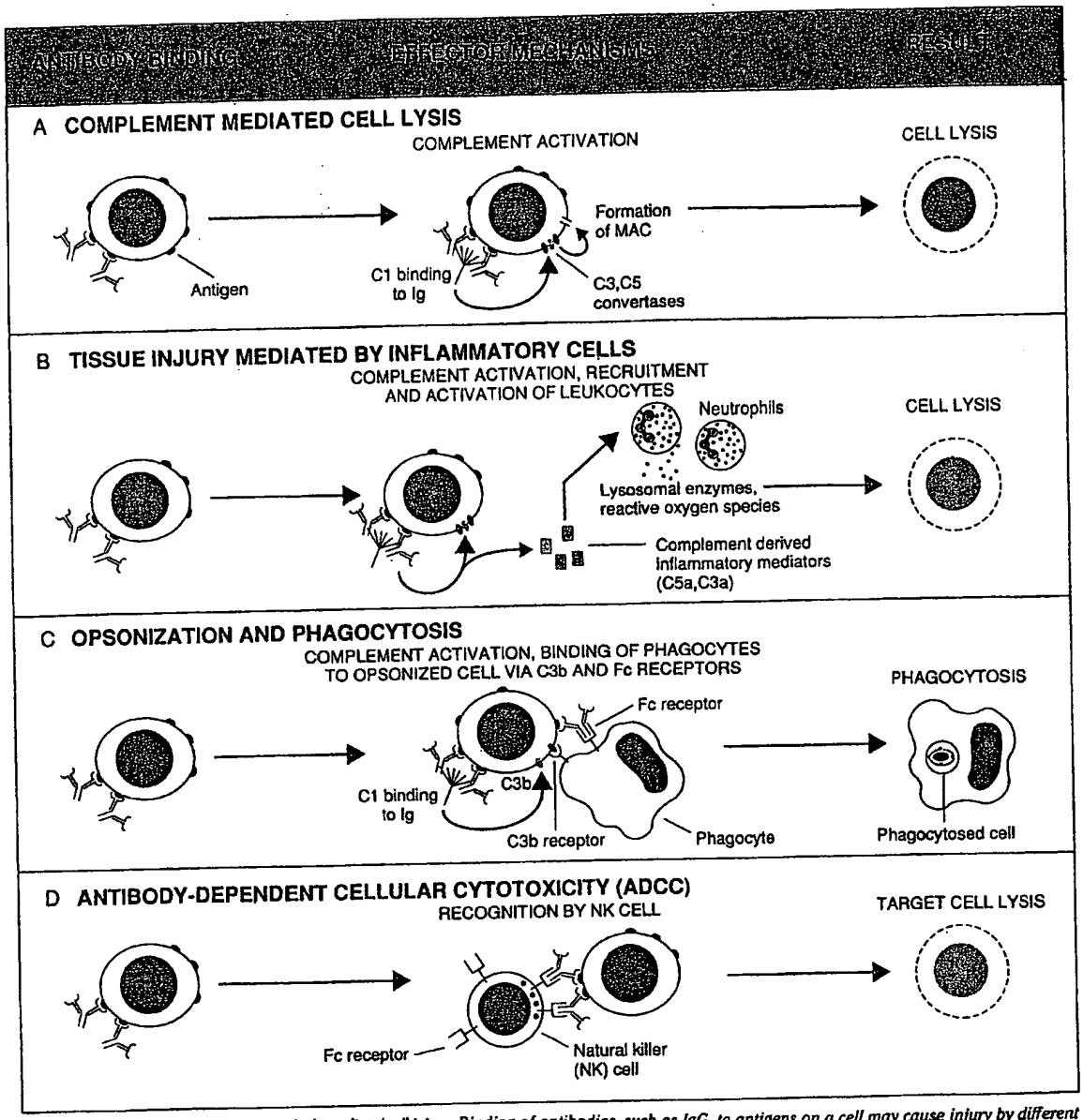


FIGURE 18-2. Effector mechanisms in antibody-mediated cell injury. *Binding of antibodies, such as IgG, to antigens on a cell may cause injury by different effector mechanisms* (A–D).
A. *Activation of complement and formation of the cytocidal membrane attack complex (MAC).*
B. *Recruitment and activation of neutrophils by complement by-products, followed by neutrophil degranulation and release of cytocidal substances.*
C. *Phagocytosis of opsonized cells by macrophages or neutrophils.*
D. *Cytolysis by natural killer (NK) cells and other leukocytes bearing Fcγ receptors.*

For instance, in autoimmune hemolytic anemia, autoantibodies are produced against self erythrocytes. The opsonized erythrocytes are phagocytosed by macrophages in the liver and spleen. This leads to depletion of the erythrocytes and hence gives rise to anemia.

4. *Lysis of antibody-coated cells by natural killer (NK) cells* (Fig. 18-2*D*) has been postulated as a mechanism for tissue injury in some diseases, such as autoimmune thyroiditis.

5. *Antibodies can cause pathologic effects by binding to functionally important molecules and altering cellular functions.* Examples of such diseases are described later in the chapter.

Immune Complex–Mediated Diseases

The occurrence of diseases due to immune complexes was suspected as early as 1911 by an astute physician named Clemens von Pirquet. At that time, diphtheria infections were being treated with serum from horses immunized with the diphtheria toxin. This is an example of passive immunization against diphtheria toxin by the transfer of serum containing anti-toxin antibodies. Von Pirquet noted that patients injected with the anti-toxin containing horse serum were developing joint inflammation (arthritis), skin rash, and fever. Two clinical features of this reaction suggested that it was not due to an infection or a toxic component of the serum itself. First, these symptoms appeared even after the injection of horse serum not containing the anti-toxin, so that the lesions could not be attributed to the anti-diphtheria antibody. Second, the symptoms appeared at least a week or so after the first injection of the horse serum and more rapidly with each repeated injection. Von Pirquet concluded that this disease was due to a host response to some component of the serum. He suggested that the host made antibodies to horse serum proteins, these antibodies formed complexes with the injected proteins and the disease was due to the antibodies or immune complexes. We now know that his conclusions were entirely accurate. He called this disease "serum disease"; it is now more commonly known as **serum sickness**, and is the prototype for immune complex–mediated disorders.

Experimental Models of Serum Sickness

Much of our current knowledge of immune complex diseases is based on analysis of experimental models of serum sickness, performed in detail by Frank Dixon and his associates in the 1960s using techniques for accurately measuring the levels of antigens and antibodies in the blood and tissues. These investigators showed that if a rabbit is injected intravenously with a single dose (greater than 50 mg/kg of body weight) of a foreign protein antigen, bovine serum albumin (BSA), within a few days the rabbit begins to produce specific anti-BSA antibodies (Fig. 18–3). These antibodies complex with circulating BSA, leading to enhanced phagocytosis and clearance of the antigen by macrophages in the liver and spleen. Immune complexes are initially detected in the circulation and then deposit in tissues, where they activate complement, with a concomitant fall in serum complement levels. Complement activation leads to recruitment and activation of inflammatory cells, predominantly neutrophils, at the sites of immune complex deposition, and the neutrophils cause tissue injury. Since the complexes deposit mainly in arteries, renal glomeruli, and the synovia of joints, the clinical and pathologic manifestations are vasculitis, nephritis, and arthritis. The clinical symptoms are usually short-lived, and the lesions heal unless the antigen is injected again. This type of disease is an example of **acute serum sickness.** It is produced by the administration of a single large dose of a foreign antigen and is characterized by the deposition of large immune complexes. A more chronic disease, called **chronic**

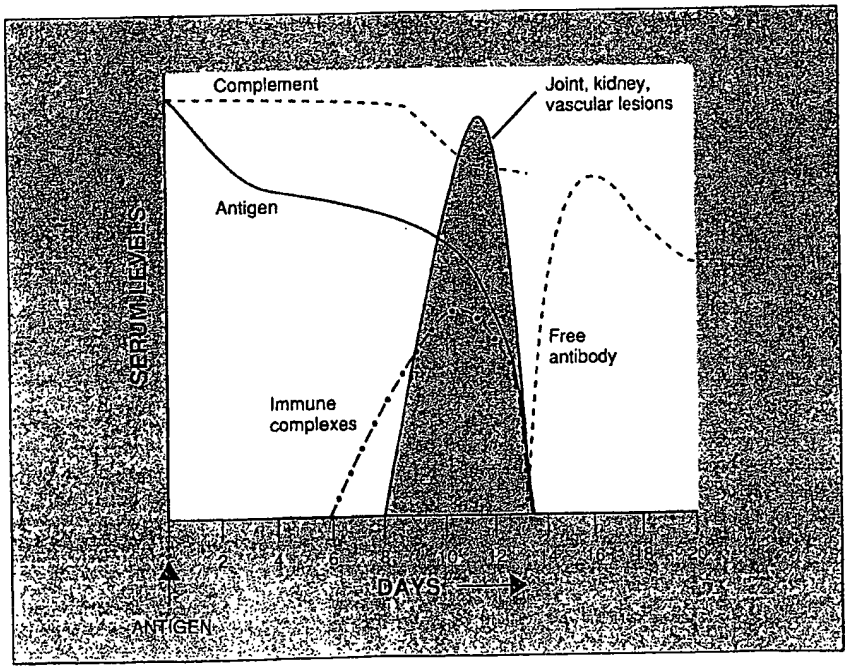


FIGURE 18–3. Sequence of immunologic responses in experimental acute serum sickness. Injection of bovine serum albumin into a rabbit leads to the production of specific antibody and the formation of immune complexes. These complexes deposit in tissues, activate complement (leading to a fall in serum complement levels), and cause lesions, which resolve as the complexes as well as the remaining antigen are removed. (Adapted with permission from Cochrane, C. G. Immune complex-mediated tissue injury. In *Cohen, S., P. A. Ward, and R. T. McCluskey (eds.). Mechanisms of Immunopathology. New York, John Wiley & Sons, Inc., 1979, pp. 29–48.)*

serum sickness, is produced by multiple injections of antigen, which lead to the formation of smaller complexes that deposit most often in kidneys, arteries, and lungs.

A localized form of experimental immune complex–mediated vasculitis is called the **Arthus reaction.** It is induced by injecting an antigen subcutaneously into a previously immunized animal. The animal contains circulating antibodies that bind to the injected antigen, forming immune complexes that deposit in the walls of small arteries at the injection site. This gives rise to a local cutaneous vasculitis with necrosis. As we shall discuss later, various diseases in humans are believed to be the clinical counterparts of acute and chronic serum sickness and the Arthus reaction.

Factors That Influence Immune Complex Deposition

From analyses of these experimental models of immune complex–mediated diseases, it is now known that several factors determine the extent of immune complex deposition.

1. The *size of circulating immune complexes* is a major factor, because very small complexes are not deposited and large ones are phagocytosed by mononuclear phagocytes and cleared. Usually, small and intermediate-sized immune complexes are prone to tissue deposition, but this may vary with different combinations of antigens and antibodies.

2. The extent of immune complex deposition in tissues is inversely proportional to the *ability of the host to clear immune complexes from the circulation.* Removal of circulating immune complexes is determined by the functional integrity of the mononuclear phagocyte system and the binding of complement proteins, which function to enhance the clearance of the complexes. Defective phagocytosis may promote the persistence and subsequent tissue deposition of immune complexes. In patients with genetic deficiencies of proteins of the classical complement pathway, such as C2 and C4 (see Chapter 13), immune complex-mediated diseases often develop, because defective production of C3b by antigen-antibody reactions and the absence of complement receptor-mediated phagocytosis lead to persistence of immune complexes in the blood. In this situation, immune complexes that deposit in tissues presumably recruit inflammatory cells by complement-independent mechanisms or by activating the alternative complement pathway.

3. The *physicochemical properties of antigens and antibodies*, including charge, valence, avidity of interaction, and Ig isotype, may influence immune complex formation and deposition. For instance, complexes containing cationic antigens bind avidly to negatively charged components of the basement membranes of kidney glomeruli. Such complexes typically produce severe and long-lasting tissue injury.

4. *Anatomic and hemodynamic factors* are important determinants of the sites of immune complex deposition. Capillaries in renal glomeruli and synovia are vessels in which plasma is ultrafiltered (to form urine and synovial fluid, respectively) by passing through the capillary wall at high hydrostatic pressure, and these are among the most common sites of immune complex deposition.

5. Finally, it is thought that immune complexes bind to inflammatory cells and stimulate *local secretion of cytokines and vasoactive mediators*, which cause increased vascular permeability and enhanced deposition of immune complexes in vessel walls by enlarging interendothelial spaces. This may lead to amplification of tissue injury and disease.

Role of Immune Complexes in Tissue Injury and Disease

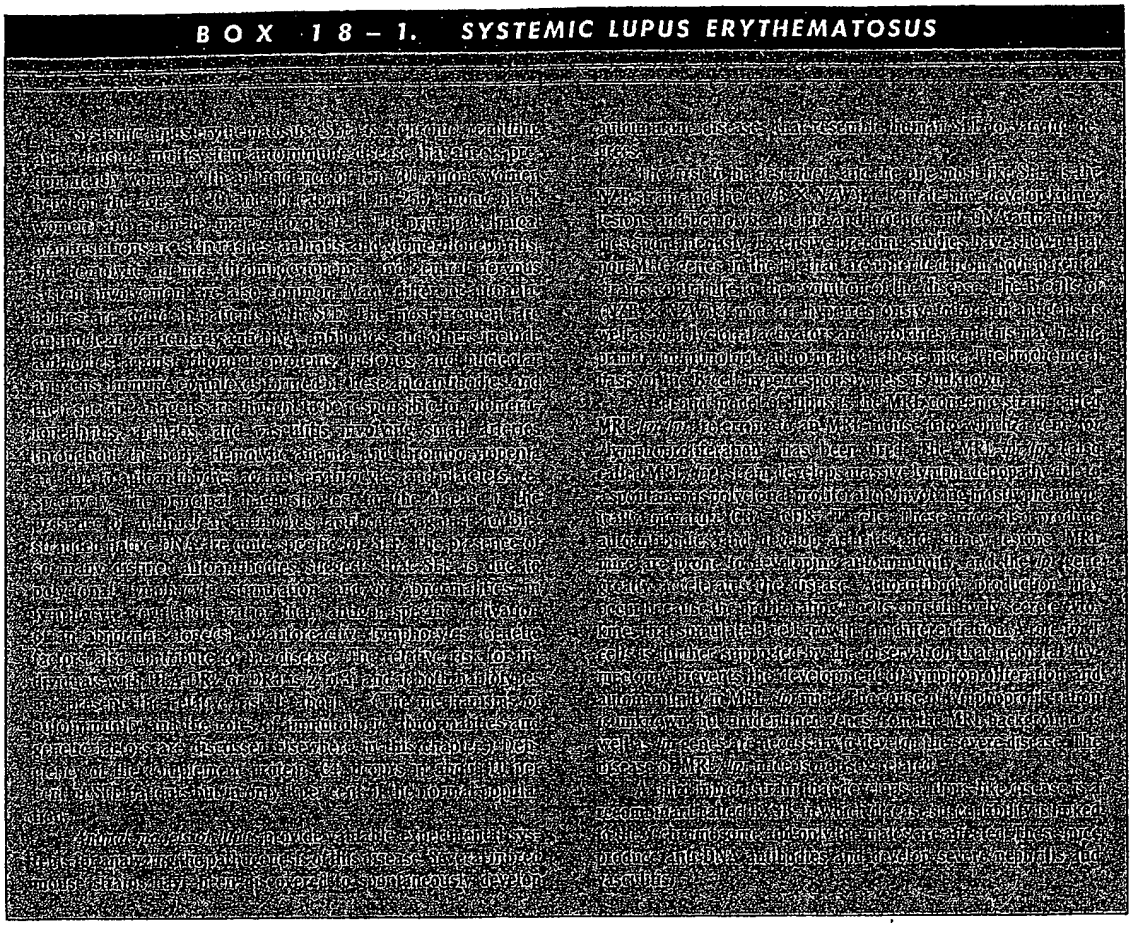
It is likely that antigen-antibody complexes are produced during many immune responses but are of pathologic significance only if the quantity, structure, or clearance of the complexes or local functional and anatomic properties is such that abnormally large amounts are deposited in tissues. The *morphologic hallmarks of immune complex–mediated tissue injury are* (1) *necrosis*, which often contains fibrin because of leakage of plasma proteins and is also called *"fibrinoid necrosis," and* (2) *cellular infiltrates composed predominantly of neutrophils.* Irregularly shaped (granular) deposits of antibody and complement components can be detected in these tissues by immunofluorescence, and if the antigen is known, it is possible to also identify antigen molecules in the deposits.

There is compelling evidence supporting a primary *pathogenic role of immune complexes in many human systemic immunologic diseases* (Table 18–2).

TABLE 18–2. Examples of Human Immune Complex Diseases

Disease	Antigen	Antibody	Clinicopathologic Manifestations
Post-streptococcal glomerulonephritis	Streptococcal cell wall antigens(s)	Anti-streptococcal antibody	Nephritis with glomerular lesions
Systemic lupus erythematosus	DNA, nucleoproteins, others	Autoantibodies (various)	Nephritis, arthritis, vasculitis (disseminated)
Polyarteritis nodosa	Hepatitis B surface antigen (HBs Ag)	Anti-HBs antibody	Arteritis (disseminated)

BOX 18–1. SYSTEMIC LUPUS ERYTHEMATOSUS



Systemic lupus erythematosus (SLE) (Box 18–1) is an autoimmune disease in which numerous autoantibodies are produced. Its many clinical manifestations include glomerulonephritis and arthritis, which are attributed to the deposition of immune complexes composed of self DNA or nucleoprotein antigens and specific antibodies (Fig. 18–4). The glomerular lesions of SLE often resemble the lesions seen in chronic serum sickness. Some cases of a form of systemic vasculitis called **polyarteritis nodosa** occur as a late sequel of hepatitis B virus infection and are due to arterial deposition of immune complexes composed of hepatitis virus surface antigen (HBsAg) and specific antibodies. **Post-streptococcal glomerulonephritis** is a kidney disease that develops 1 to 3 weeks after streptococcal skin and throat infections. It is thought to be due to glomerular deposits of immune complexes composed of streptococcal antigen and anti-streptococcal antibodies. However, post-streptococcal glomerulonephritis differs from typical immune complex–mediated diseases because it involves only the kidneys and there are no systemic manifestations. It is, therefore, possible that this disease may be due to initial binding of streptococcal antigen to glomeruli followed by deposition of the antibody, so that the pathogenic mechanism is not deposition of circulating complexes but free antibody against a "planted" bacterial antigen. Granular deposits of antibody and complement have been demonstrated in injured tissues in many other forms of cutaneous necrotizing vasculitis, arthritis and glomerulonephritis. Some skin diseases associated with vasculitis are morphologically similar to experimental Arthus reactions. Such diseases are postulated to be due to immune complexes, but the nature of the antigens is unknown.

Diseases Mediated by Antibodies Against Fixed Cell and Tissue Antigens

As we mentioned earlier, antibodies produced against fixed cellular or tissue antigens are usually autoantibodies. Less frequently, the antibodies may

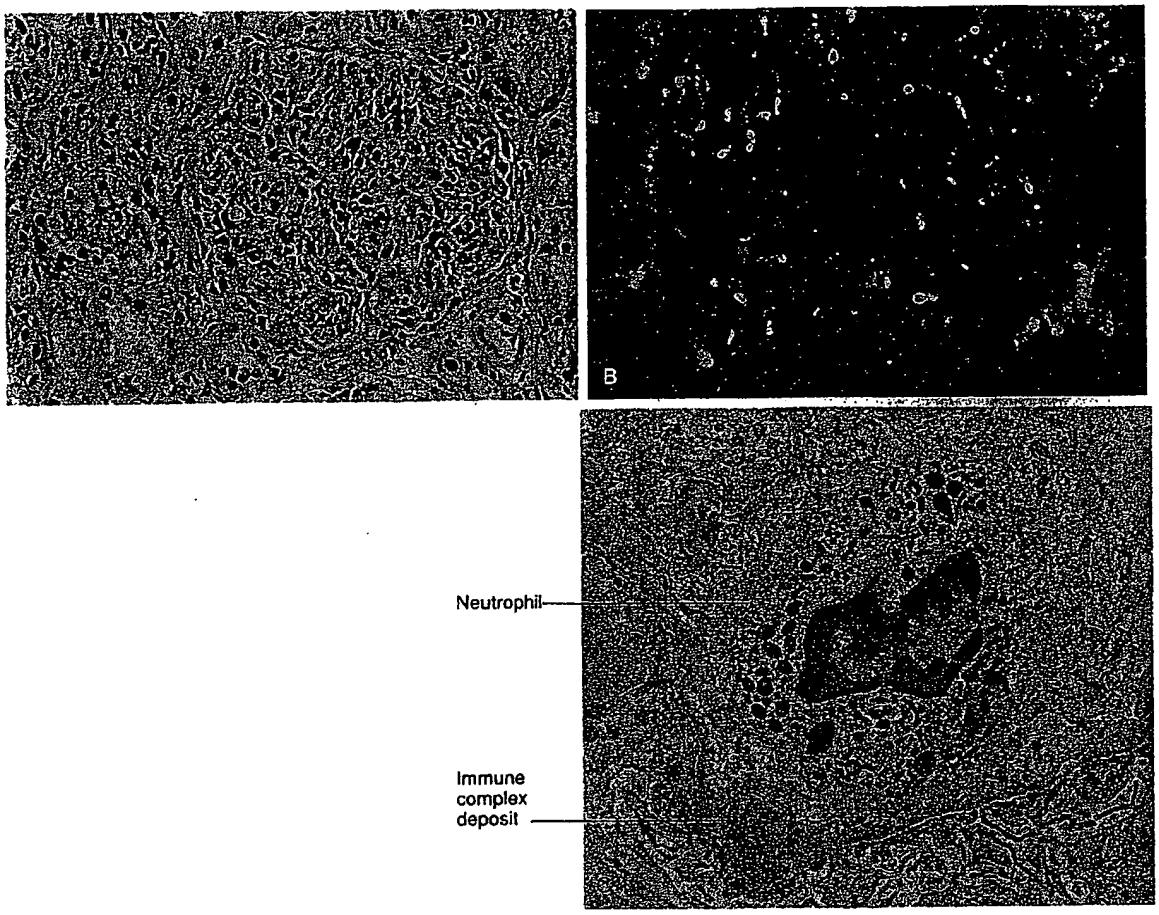


FIGURE 18–4. Histopathology of immune complex–mediated glomerulonephritis.
A. *Light micrograph of a kidney glomerulus, showing hypercellularity caused by infiltration of leukocytes.*
B. *Immunofluorescent stain for IgG showing granular deposits in a glomerulus. (Complement proteins, including C3b, would co-localize with the antibody.)*
C. *Electron micrograph of a glomerular capillary, showing an immune complex deposited in the wall and a neutrophil in the lumen.*
(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston; reproduced with permission from Brenner, B. M., F. L. Coe, and F. C. Rector. Clinical Nephrology, Philadelphia, W.B. Saunders Co., 1987.)

be produced against extrinsic antigens but may bind to immunologically similar or cross-reactive antigens present in autologous cells or tissues. Antibodies against self tissues are not always pathogenic. For instance, tissue injury due to ischemia or infection may lead to autoantibody production because of alterations of self antigens or exposure of antigens that are normally sequestered from the immune system. In such situations, the autoantibodies may be the result and not the cause of tissue necrosis. Some patients who suffer myocardial infarctions develop, within a few weeks, antibodies against their own cardiac cells. Obviously, the autoantibodies are not the cause but the result of the infarction.

Many tissue or organ-specific immunologic diseases are associated with the production of, and are thought to be caused by, autoantibodies (Table 18–3). In most of these diseases, specific circulating antibodies can be found in the blood but the mechanisms responsible for autoantibody production are not known. Autoimmune hemolytic anemia and immune thrombocytopenia are due to autoantibodies against erythrocytes and platelets, respectively. The antibodies cause complement-dependent lysis of the circulating cells and opsonize the cells leading to enhanced phagocytosis by mononuclear phagocytes. Autoimmune hemolytic anemia and thrombocytopenia are usually idiopathic and may sometimes be associated with other immunologic abnormalities, e.g., in SLE. Similar diseases occur during idiosyncratic reactions to some drugs and may be due to binding of the drugs to cell surfaces, leading to the creation of neo-antigens which elicit specific antibody responses. **Goodpasture's syndrome** is a disease characterized by lung

TABLE 18-3. Examples of Autoantibodies in Human Diseases

Disease	Principal Clinical Features	Autoantibody Detected: Specificity	Method of Detection
Glomerulonephritis (Goodpasture's syndrome)	Nephritis with proteinuria, renal failure; lung hemorrhages	Type IV collagen in basement membranes of kidney glomeruli and lung alveoli	Immunofluorescence
Autoimmune hemolytic anemia	Hemolysis, anemia	Erythrocyte membrane proteins	Hemagglutination
Autoimmune thrombocytopenic purpura	Platelet deficiency (thrombocytopenia), bleeding disorders	Platelet membrane proteins (e.g., gp IIb/IIIa)	Immunofluorescence
Pemphigus vulgaris	Decreased adhesions between epidermal keratinocytes; skin vesicles (bullae)	Intercellular junctions of epidermal cells	Immunofluorescence
Bullous pemphigoid	Detachment of epidermal cells; skin vesicles	Epidermal basement membrane proteins	Immunofluorescence
Myasthenia gravis	Muscle weakness	Acetylcholine receptor	Immunoprecipitation
Graves' disease (hyperthyroidism)	Hyperthyroidism due to increased production of thyroid hormones	Thyroid-stimulating hormone receptor on thyroid follicular epithelial cells	Bioassay
Insulin-resistant diabetes mellitus	Diabetes, unresponsive to insulin therapy	Insulin receptor	Inhibition of insulin binding to cultured cells
Pernicious anemia	Abnormal erythropoiesis due to vitamin B_{12} deficiency	Intrinsic factor; gastric parietal cells	Bioassay; immunofluorescence

hemorrhages and severe glomerulonephritis. It is caused by an autoantibody that binds to epitopes of type IV collagen found in the basement membranes of pulmonary alveoli and glomerular capillaries and leads to local activation of complement and neutrophils. On microscopic examination, necrosis, leukocytic infiltrates, and linear deposits of antibody and complement along basement membranes can be seen (Fig. 18-5). A number of skin diseases are due to antibodies against epidermal cells or basement membrane antigens.

Autoantibodies against cell surface receptors may lead to functional abnormalities without the involvement of any other effector mechanisms. For instance, some antibodies against cell surface hormone receptors bind to these receptors and lead to aberrations in cellular physiology without inflammation or tissue injury. These functional abnormalities may result from receptor-mediated stimulation of target cells or inhibition due to interference with receptor function (Fig. 18-6).

One example of stimulation by an antibody mimicking a physiologic molecule is **Graves' disease,** an autoimmune disease of the thyroid gland characterized by hyperthyroidism. The clinical syndrome results from excessive production of thyroid hormones such as thyroxine. This disease is usually caused by an autoantibody specific for the receptor for thyroid-stimulating hormone (TSH) on thyroid epithelial cells. TSH is a pituitary hormone whose normal function is to stimulate the production of thyroid hormones by thyroid epithelial cells. Binding of antibody to the TSH receptor has the same effect as TSH itself, leading to unregulated stimulation of thyroid epithelial cells and excess thyroid hormone production even in the absence of TSH.

An example of anti-receptor antibody-mediated functional inhibition is **myasthenia gravis,** a disease of progressive muscle weakness caused by autoantibodies reactive with acetylcholine receptors in the motor end plates of neuromuscular junctions. Binding of the antibodies interferes with acetylcholine mediated neuromuscular transmission and may lead to a reduction in receptor numbers as a consequence of endocytosis and intracellular degradation ("down-modulation") of the receptors. The result is a failure of muscle to respond to normal neural impulses, leading to progressive muscle weakness. Experimentally, a disease resembling myasthenia gravis can be produced in rats and mice by immunizing them with purified acetylcholine receptors. The experimental disease can be adoptively transferred to normal animals by antibodies against the acetylcholine receptor. Similarly, some patients with diabetes mellitus who are unresponsive to insulin have autoantibodies against insulin receptors that block the binding and the physiologic effects of the hormone.

Autoantibodies against physiologically important circulating molecules, such as hormones, may also lead to functional abnormalities and disease in the absence of cell or tissue destruction. Some cases of **pernicious anemia** are associated with autoantibodies against intrinsic factor, which is a cofactor for the intestinal absorption of vitamin B_{12}. The antibodies are thought to

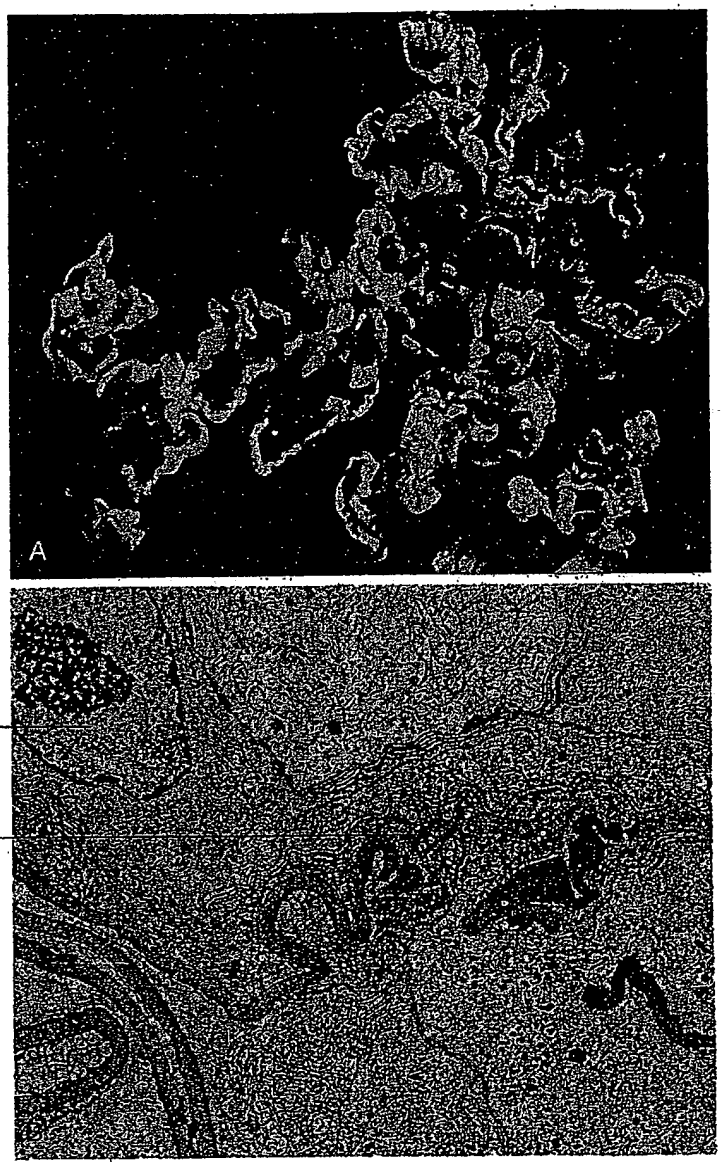


FIGURE 18–5. Pathology of glomerulonephritis induced by an antibody against the glomerular basement membrane (Goodpasture's syndrome).

A. *Immunofluorescent stain for IgG, showing linear deposition of antibody along the capillary basement membrane of a glomerulus. (This pattern is very different from that of immune complex-mediated diseases; see Fig. 18-4B.)*

B. *Electron micrograph of a glomerular capillary, showing destruction of the basement membrane without evidence of immune complex deposition.*

(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston; reproduced with permission from Brenner, B. M., F. L. Coe, and F. C. Rector. Clinical Nephrology. Philadelphia, W.B. Saunders Co., 1987.)

bind to and inhibit the function of intrinsic factor, resulting in vitamin B_{12} deficiency. This causes abnormal hematopoiesis and megaloblastic anemia.

Some human diseases are also due to antibodies produced against foreign antigens that cross-react with self proteins. Perhaps the best example is **acute rheumatic fever,** which, like post-streptococcal glomerulonephritis, is a late sequela of throat infection caused by streptococci. The bacterial strains associated with rheumatic fever are usually different from those that lead to glomerulonephritis. Rheumatic fever is characterized by arthritis, endocarditis resulting in lesions of heart valves, myocarditis, and neurologic abnormalities, but no kidney abnormalities. The myocardial injury is thought to be due to an antibody against a streptococcal cell wall protein that binds to a cross-reactive antigen in cardiac muscle cells.

Despite the numerous examples of circulating autoantibodies associated with immunologic diseases, it is important to reiterate that it is often not clear whether a particular antibody is the cause of the disease or is produced as a result of cell or tissue injury. Furthermore, autoantibodies may be present but may

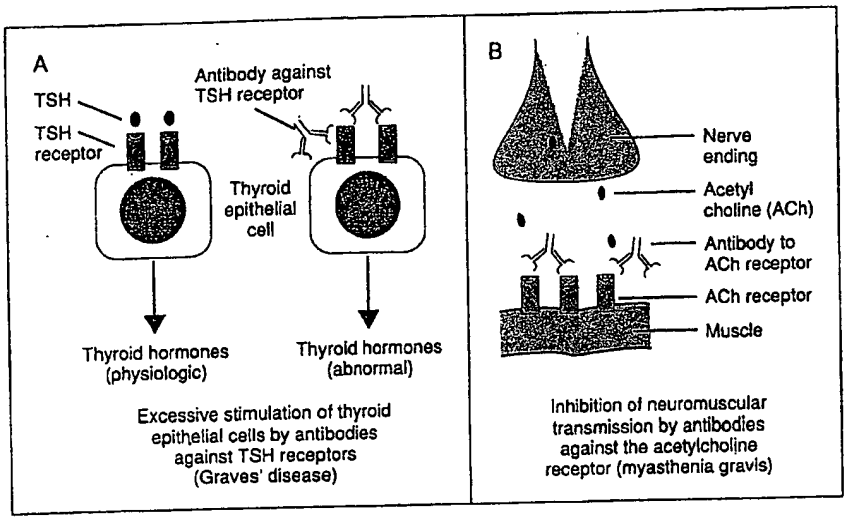


FIGURE 18–6. Effector mechanisms in antibody-mediated diseases: functional abnormalities induced by antibodies against hormone receptors.

A. *Antibodies against thyroid-stimulating hormone (TSH) receptors stimulate thyroid epithelial cells by binding to the receptor and by mimicking the effects of TSH, the physiologic ligand.*

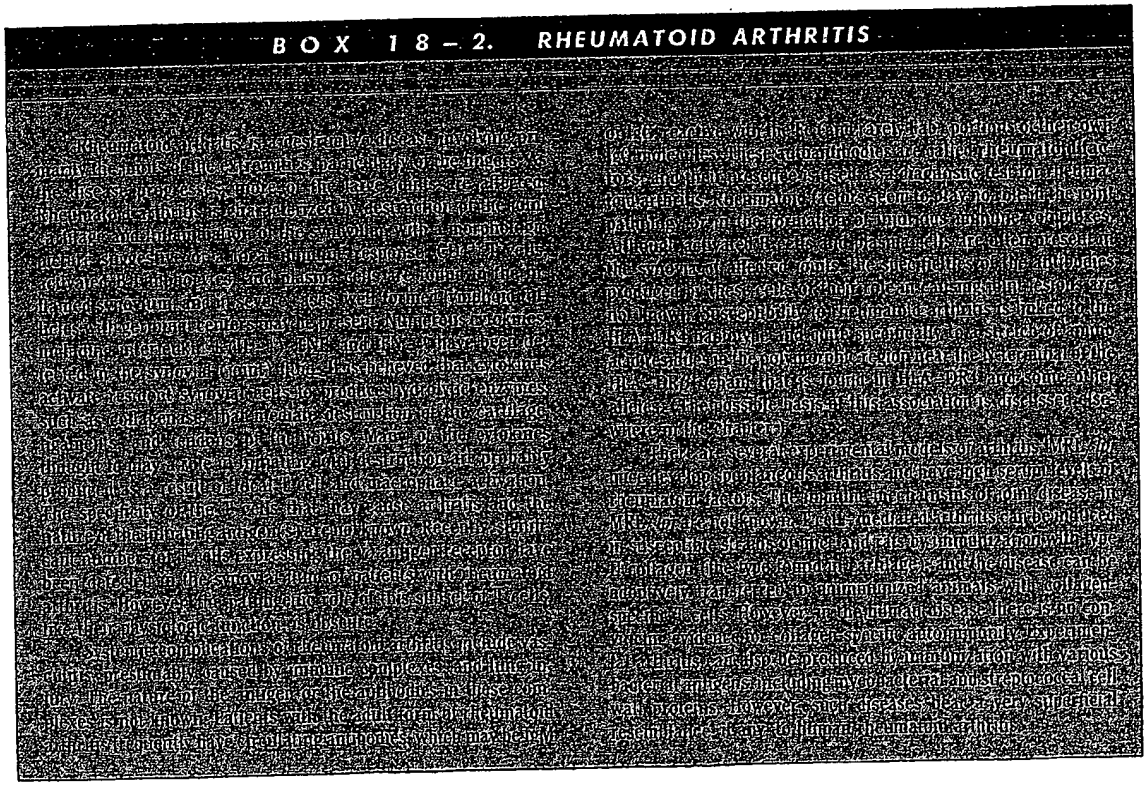
B. *Antibodies against the acetylcholine (ACh) receptor inhibit neuromuscular transmission by binding to the ACh receptor, leading to down-modulation of receptors and competitive inhibition of ACh binding.*

not be responsible for pathologic abnormalities. For instance, patients with **rheumatoid arthritis** (Box 18–2) frequently have a circulating IgM antibody that is specific for their own IgG molecules, usually the Fc portions of these molecules. Such autoantibodies are called rheumatoid factors. Although their presence is a useful diagnostic test for rheumatoid arthritis, there is no evidence that rheumatoid factors are involved in the formation of injurious immune complexes or contribute to the joint lesions in this disease.

DISEASES CAUSED BY T CELLS

The potential importance of T lymphocytes as mediators of human immunologic diseases has been

BOX 18–2. RHEUMATOID ARTHRITIS



increasingly recognized in the 1980s. This is largely because of two technological advances—the production of monoclonal antibodies that identify phenotypically and functionally distinct subsets of T cells, and methods for isolating and propagating T cells from lymphoid tissues and lesions. As we shall see later in this chapter, the demonstration that T lymphocytes are critical for maintaining self-tolerance to many protein antigens has led to increasing interest in their role in autoimmune disorders.

Mechanisms of T Cell–Mediated Tissue Injury

The T cells that cause tissue injury may be autoreactive, or they may be specific for foreign protein antigens that are present in or bound to one's own cells or tissues. The pathologic lesions vary, depending on the types of T cells that produce these lesions. T cells injure tissues by the same two mechanisms that are responsible for cell-mediated immunity against microbes (see Chapter 12):

1. T cells, usually of the CD4+ subset, secrete cytokines, which activate macrophages, giving rise to **DTH reactions.** Acute tissue injury results from the products of activated macrophages, such as hydrolytic enzymes and toxic oxygen metabolites. Chronic DTH reactions often produce fibrosis as a result of the secretion of cytokines and growth factors by the macrophages (see Chapter 12).
2. CD8+ **cytolytic T lymphocytes** (CTLs) directly lyse target cells bearing class I major histocompatibility complex (MHC)–associated foreign antigens, without the participation of macrophages or any other effector mechanisms.

A role for T cells in causing a particular immunologic disease is suspected largely by the demonstration of T cells in lesions and by the isolation of T cells specific for self antigens from the tissues or blood of patients. Furthermore, cytokines secreted by activated T cells induce alterations in adjacent tissues that are used as indicators of local T cell stimulation. One such cytokine is γ-interferon (IFN-γ), which induces the expression of class II MHC molecules on vascular endothelium as well as on other cells that do not express these molecules constitutively. Abnormal expression of class II MHC molecules in a tissue suggests that T cells have been activated in the immediate environment. Aberrant expression of class II MHC molecules may also lead to excessive T cell activation, because many more cells may acquire the ability to present antigen. However, epithelial and mesenchymal cells that are induced to express class II molecules may not produce costimulators that are necessary for T cell activation and, therefore, may not be efficient at stimulating T cells (Chapter 7).

The presence of activated T cells in the blood or tissues of patients is not always associated with disorders of cell-mediated immunity. For instance, CD4+ T cells function as helper cells in humoral immunity and, therefore, may be abundant in lesions that are mediated by antibodies and not by the T cells themselves. Moreover, as for antibodies, the identification and even isolation of T cells are not, by themselves, proof of their pathogenic role. The most definitive proof is the ability to adoptively transfer the disease to normal recipients, and this is not possible in the clinical situation. However, in experimental models of several immunologic diseases, the lesions have been transferred to normal syngeneic animals by purified T cells or by antigen-specific cloned lines of T cells (Table 18–4). The similarities between these experimentally induced lesions and clinical diseases further support a primary pathogenic role of T cells in the latter. Finally, alterations in the ratio of circulating CD4+ and CD8+ T cells (the normal being about 2:1) have been used as diagnostic indices for T cell–mediated immunologic diseases. Such assays, however, are of limited usefulness because they are not specific for particular immunologic abnormalities.

TABLE 18–4. Identification of Antigen-Specific T Cells in Immunologic Diseases

Disease	Specificity of T Cell Clone/Line	Isolated from Lesions or Blood of		Ability to Transfer Disease in Animal Models
		Patients	*Animal Models*	
Experimental allergic encephalomyelitis	Myelin basic protein	No	Yes	Yes
Experimental allergic neuritis	P2 protein of peripheral nerve myelin	No	Yes	Yes
*Myasthenia gravis**	Acetylcholine receptor	Yes	Yes	Yes
*Some cases of Graves' disease,** *autoimmune thyroiditis*	Thyroid follicular epithelial cells	Yes	Yes	Yes
Viral myocarditis	Viruses (e.g., Coxsackie)	No	Yes†	Yes

* In these cases, the T cells may be helper cells that stimulate local production of autoantibodies, which are responsible for inducing lesions.

† $CD8^+$ cytolytic T lymphocyte (CTL) clones; pathogenic T cells in the other examples listed are $CD4^+$ cells.

Diseases Caused by CD4+ T Cells and Delayed Type Hypersensitivity

A variety of cutaneous diseases that result from topical exposure to foreign antigens or are sequelae of skin infections are due to CD4+ T cell-mediated DTH reactions. These include skin rashes as a result of *contact sensitivity to chemicals*, such as drugs, cosmetics, and environmental antigens. The rashes usually appear hours or even days after exposure to the contact sensitizing agent. The lesions may be due to T cell responses to neo-antigens created by binding of the chemicals to normal cell surface proteins on epidermal keratinocytes or Langerhans cells. Skin biopsy specimens show dermal perivascular infiltrates of lymphocytes and macrophages plus edema and fibrin deposition resulting from leakage of plasma from dermal capillaries and venules (see Chapter 12, Fig. 12-2). Vascular endothelial cells in the lesions may express enhanced levels of cytokine-regulated surface molecules, such as class II MHC molecules. These DTH reactions are quite different mechanistically and morphologically from two other types of immunologic skin lesions, IgE-mediated immediate hypersensitivity and immune complex-mediated Arthus reactions (Table 18-5).

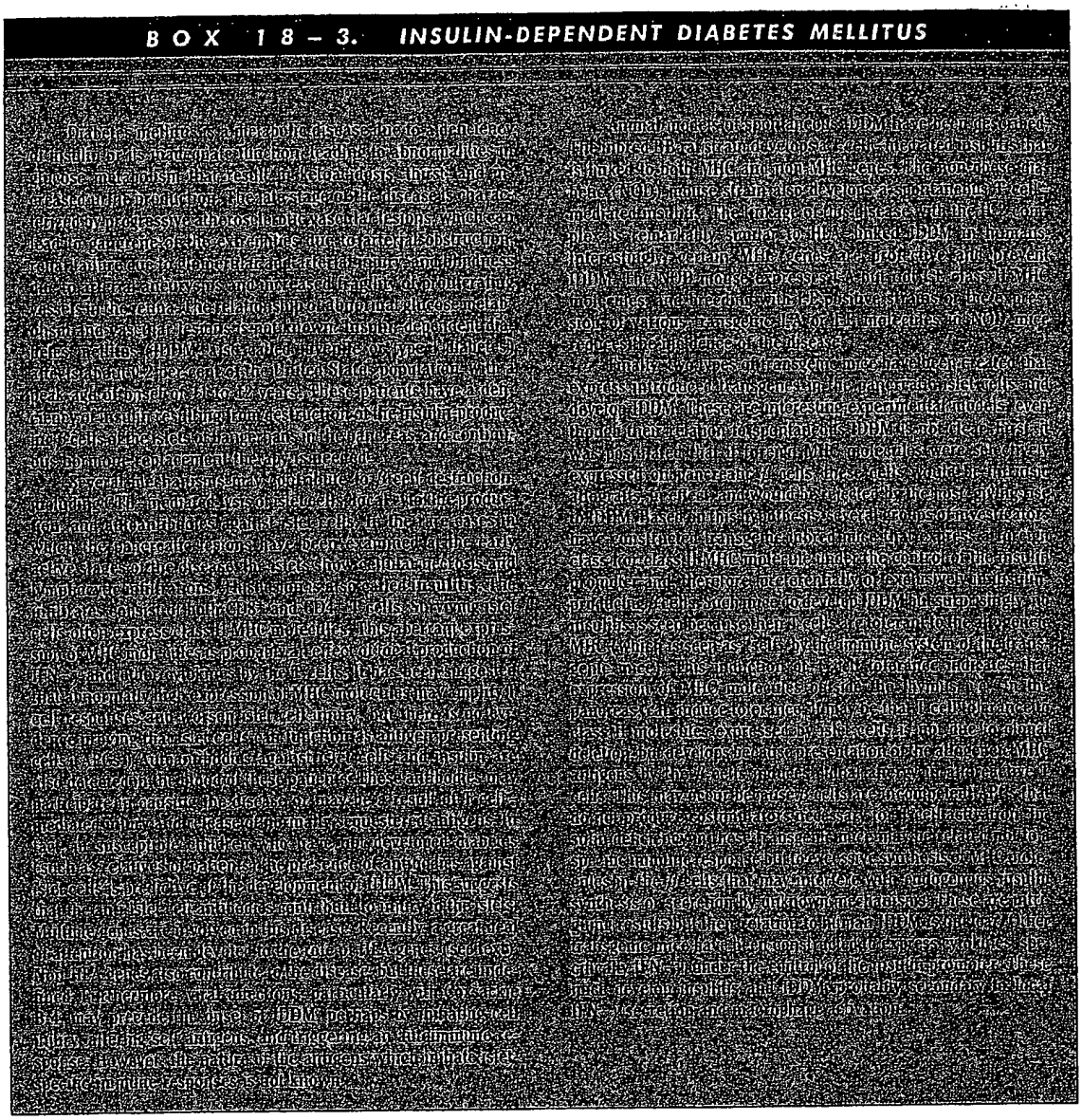
A number of organ-specific autoimmune diseases are thought to be caused by autoreactive T cells. In some patients with **insulin-dependent diabetes mellitus** (IDDM) (Box 18-3), there are infiltrates of lymphocytes and macrophages around islets of Langerhans in the pancreas, with destruction of insulin-producing β cells in the islets and a resultant deficiency in insulin production. Residual islet cells in these lesions express class II MHC molecules, again suggesting local cytokine production. Similar findings have been observed in spontaneous diabetes in rats and mice, in which the lesions have been adoptively transferred to normal animals with CD4+ T cells from diseased animals. CD8+ CTLs may also contribute to insulitis and islet cell destruction in human and experimental diabetes mellitus. The specificity of the T cells that cause insulitis and destroy islet cells, and the nature of the initiating antigen, are unknown. **Experimental allergic encephalomyelitis** (EAE) (Box 18-4) is a neurologic disease that can be induced in experimental animals by immunization with myelin basic protein in adjuvant. Such immunization leads to an autoimmune T cell response against myelin, culminating in activation of macrophages around nerves in the brain and spinal cord, destruction of the myelin, abnormalities in nerve conduction, and neurologic deficits. EAE can be transferred to naive animals with myelin basic protein-specific CD4+ T cells, and the experimental disease can be blocked by antibodies specific for class II MHC or for CD4 molecules, indicating that CD4+ class II MHC-restricted T cells play an obligatory role in this disorder. A role for CTLs in this experimental disease is also suspected but remains unproved. It has

TABLE 18-5. Lesions and Mechanisms of Different Forms of Immunologic Reactions in the Skin

	Immediate Hypersensitivity	Immune Complex-Mediated Injury	Delayed Type Hypersensitivity
Induced by	Antigens that evoke IgE response (genetic predisposition?)	Antigens that induce IgM, IgG antibodies	Protein antigens; chemicals that bind to self proteins
Form of Cutaneous Reaction	Urticaria, wheal	Arthus reaction	Contact sensitivity, tuberculin reaction
Onset After Antigen Challenge	Minutes*	Usually 2-6 hours	Usually 24-48 hours
Pathologic Lesion	Edema, vascular dilatation, local smooth muscle contraction	Necrotizing vasculitis	Perivascular cellular infiltrates and edema
Transferred to Normal Animals by	Serum	Serum	Lymphocytes
Antibody Involved	IgE	IgG (usually complement-fixing subclasses), IgM	None
Effector Cells	Mast cells with IgE bound to Fc receptors	Neutrophils, monocytes (recruited by complement-dependent and complement-independent mechanisms)	CD4+ T cells, macrophages (activated by cytokines)
Secreted Mediators, Effector Molecules	Mast cell-derived mediators: vasoactive amines, lipid mediators	Products of complement activation: membrane attack complex, C3a, C5a	Cytokines, particularly IFN-γ and TNF

* Note that the late phase reaction of immediate hypersensitivity resembles delayed type hypersensitivity.
Abbreviations: Ig, immunuglobulin; IFN, interferon.

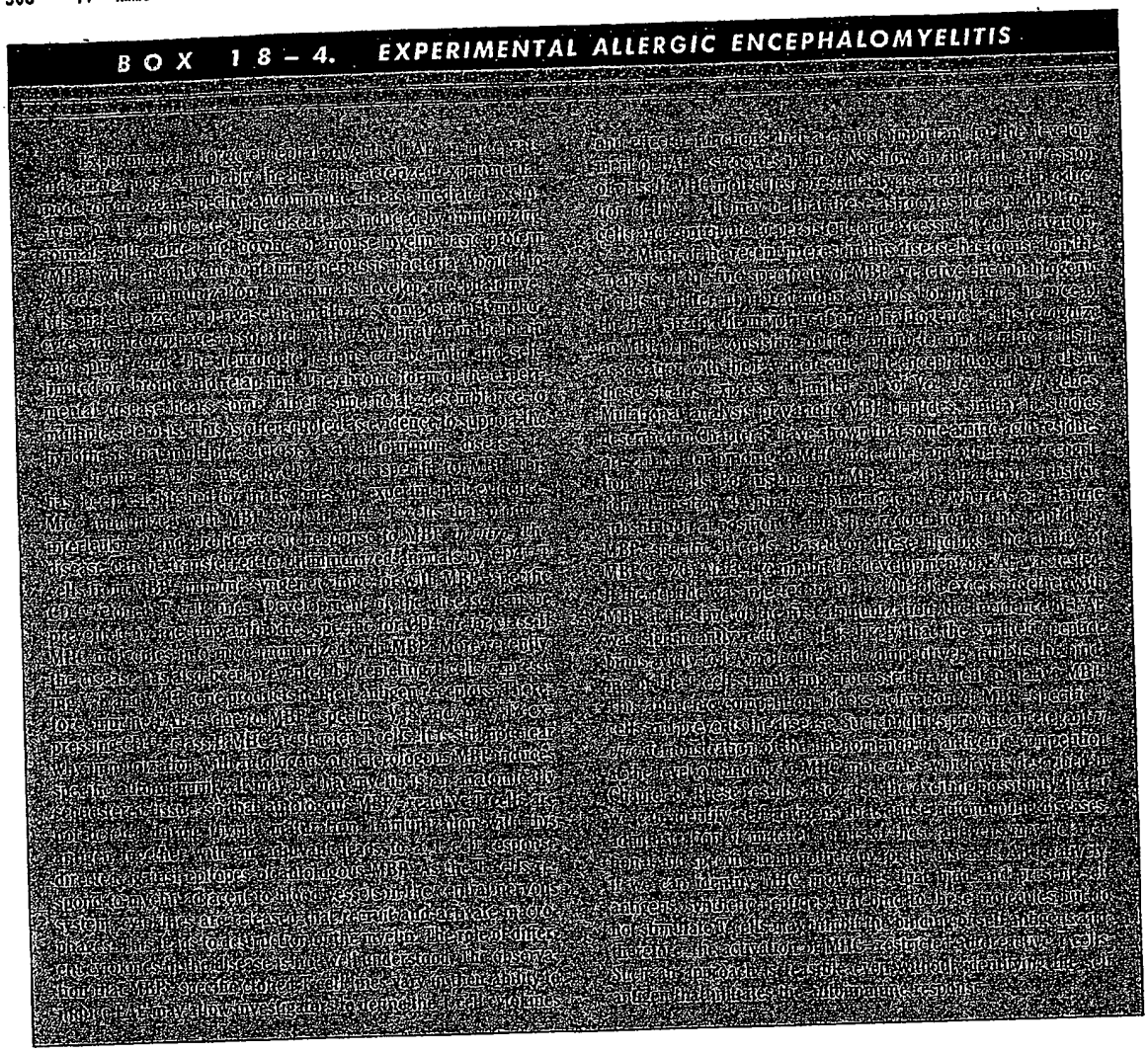
BOX 18–3. INSULIN-DEPENDENT DIABETES MELLITUS



been postulated that EAE is an experimental counterpart of a progressive neurologic disease called multiple sclerosis.

Cell-mediated immune responses to microbes and other foreign antigens may also lead to considerable injury of the tissues at the sites of infection or antigen exposure. Intracellular bacteria such as *Mycobacterium tuberculosis* induce strong T cell and macrophage responses, resulting in the formation of granulomas, and fibrosis due to the production of cytokines which stimulate fibroblast proliferation and collagen synthesis (described in Chapter 12). Therefore, mycobacterial infections often result in extensive tissue destruction and scarring that can cause severe functional impairment, for instance in the lungs. Sarcoidosis is a disease of unknown etiology in which granulomas develop in the lungs, lymphoid tissues, liver, and spleen. This disease is probably due to a T cell–mediated immune response to a foreign antigen that has eluded identification.

BOX 18–4. EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS



Diseases Caused by Cytolytic T Lymphocytes

The principal physiologic function of CTLs is to eliminate intracellular microbes, primarily viruses. It follows, therefore, that infected cells are lysed during CTL-mediated protective immune responses. Some viruses directly injure infected cells, or are cytopathic, whereas others are not. Since CTLs cannot *a priori* distinguish between cytopathic and non-cytopathic viruses, they will lyse virally infected cells whether or not the infection itself is harmful to the host. *Therefore, CTL responses to viral infections can lead to tissue injury even if the virus itself has no pathologic effects.* Examples of viral infections in which the lesions are due to the host CTL response and not the virus itself include lymphocytic choriomeningitis in mice and viral hepatitis in humans (see Chapter 15).

To date, there are few documented examples of autoimmune diseases mediated by CTLs. In mice infected with the Coxsackie B virus, myocarditis develops, with infiltration of the heart by $CD8^+$ T cells. These animals contain virus-specific, class I MHC-restricted CTLs as well as CTLs that lyse uninfected myocardial cells. It is postulated that the heart lesions are initiated by the virus infection and virus-specific CTLs, but myocardial injury leads to the exposure or alteration of self antigens and the development of autoreactive CTLs. As mentioned above, CTLs may also contribute to tissue injury in many of the disorders that are caused primarily by $CD4^+$ T cells, such as insulitis and EAE. It is likely, however, that extracellular tissue antigens preferentially stimulate $CD4^+$ T cells and that $CD8^+$ CTLs recognize and respond to endogenously synthesized antigens, such as viral proteins. One would, therefore, predict that these two forms of T cell-mediated immunity would be in-

volved in pathologic immunity against distinct types of antigens.

So far in this chapter, we have discussed the various immunologic mechanisms that cause tissue injury and disease. We have also described how immunologic diseases can result from responses to autologous antigens, foreign antigens, or foreign antigens that cross-react with self molecules. In the remainder of this chapter, we discuss the mechanisms that might lead to autoimmunity, which remains the most frequent, the least understood, and the most intensively investigated etiology for human immunologic diseases.

Mechanisms of Autoimmunity

The possibility that an individual's immune system can react against autologous antigens and lead to pathologic tissue injury was appreciated by immunologists from the time that the specificity of the immune system for foreign antigens was recognized. In the early 1900s, Paul Ehrlich coined the rather melodramatic phrase, "horror autotoxicus," for immunity against self. When Macfarlane Burnet proposed the clonal selection hypothesis 50 years later, he added the corollary that clones of autoreactive lymphocytes were deleted during development in order to prevent autoimmune reactions. The ability to discriminate between self and non-self has been emphasized throughout this book as an essential and unique property of the normal immune system. As discussed in Chapters 8 and 10, self-tolerance is due to two principal mechanisms: clonal deletion and clonal anergy.

The most effective mechanism of self-tolerance is the deletion of self-recognizing T and B lymphocytes prior to their maturation to functional competence, because of which normal individuals lack lymphocytes capable of recognizing many autologous antigens. Clonal deletion, however, cannot account for all self tolerance, because autoreactive B cells can be induced to secrete autoantibodies by polyclonal activators and not all self antigens may be present in the thymus to delete developing T cells. The alternative mechanism by which lymphocytes become self-tolerant is clonal anergy, induced by encounter with self antigens so that the self-reactive cells survive but cannot respond to these antigens. Other mechanisms of self-tolerance that have been postulated include suppressor T cells specific for self antigens, but their existence and physiologic role are unproved.

Autoimmunity results from a breakdown or failure of the mechanisms that are normally responsible for maintaining self-tolerance. Failure of self-tolerance can be due to incomplete deletion of self-reactive clones or to aberrant stimulation or regulation of self-reactive lymphocytes that are normally anergic to self antigens. Much of our knowledge of autoimmunity is based on experimental models, in which autoimmune diseases occur spontaneously or are induced by particular immunizations. Several important general concepts have emerged from the analyses of these models during the last 20 years or so:

1. *Multiple interacting factors contribute to the development of autoimmune disease.* These include immunologic abnormalities, genetic backgrounds that predispose to autoimmunity, and microbial infections that often precede clinical autoimmune diseases and may lead to aberrant lymphocyte stimulation. Because various combinations of these factors may be operative in different disorders, it is not surprising that autoimmune diseases comprise an extraordinarily heterogeneous group of clinical and pathologic abnormalities.

2. *Different types of antigens and immunologic mechanisms may cause systemic and organ-specific autoimmune diseases.* For instance, immune responses to widely disseminated antigens (such as autologous DNA in SLE) and the formation of circulating immune complexes typically produce systemic diseases. In contrast, autoimmune responses against antigens with restricted tissue distributions lead to organ-specific or tissue-specific injury, such as insulitis in diabetes mellitus and motor end-plate lesions in myasthenia gravis. It is also likely that systemic diseases characterized by multiple autoimmune phenomena are due to aberrant regulation or polyclonal activation of numerous clones of lymphocytes. In contrast, organ-specific autoimmune diseases may be due to failure of self-tolerance in lymphocytes specific for one or a few tissue antigens or abnormal activation of lymphocyte clones reactive with a limited number of antigens. The possible mechanisms leading to these two classes of autoimmune reactions are described later in the chapter.

3. *Low levels of autoantibodies are stimulated in normal individuals during immune responses to foreign antigens.* Based largely on the detection of such "natural autoantibodies" in healthy individuals, it has been suggested that the potential for autoreactivity exists normally. Natural autoantibodies are usually low-affinity antibodies of the IgM class that may be generated without T cell help and do not produce tissue injury. Pathologic autoimmunity may develop if larger amounts of high-affinity autoantibodies are produced, presumably as a result of help provided by autoreactive T cells. This concept again emphasizes the importance of T cell tolerance in maintaining unresponsiveness to self antigens.

A major difficulty in defining the mechanisms of human autoimmune diseases has been the inability to identify the antigens that initiate autoimmune responses. As a result, the specific etiologies of most autoimmune diseases are not known. Recent advances in the experimental analysis of self-tolerance, and in techniques for studying the molecular basis of antigen recognition by lymphocytes, are providing new insights into the mechanisms of autoimmunity. In the following sections, we describe the immunologic, genetic, and other factors that contribute to the development of autoimmune responses, keeping in mind

that these factors are often interrelated and act in concert to give rise to pathologic autoimmunity.

LYMPHOCYTE ABNORMALITIES IN AUTOIMMUNITY

Autoimmune diseases may result from primary abnormalities of B cells, T cells, or both. Even in disorders mediated by autoantibodies, the defect may lie in helper T lymphocytes, which are necessary for the production of high-affinity antibodies. Experimental myasthenia gravis, for instance, can be adoptively transferred to normal animals with cloned lines of helper T cells specific for acetylcholine receptors, even though the disease is caused by anti-receptor antibodies. Because helper T cells play a central role in the regulation of all immune responses, and because T cell clonal deletion is an effective way of maintaining self-tolerance, much recent attention has focused on the role of T cells in autoimmunity.

Immunologic abnormalities can disrupt self-tolerance in many different ways. First, autoimmunity may develop if self-reactive clones of lymphocytes escape normal deletion mechanisms and are allowed to mature. Since the mechanisms of clonal deletion are not yet known, it is difficult to postulate how self-reactive lymphocytes may evade such deletion. As we shall discuss later, the expression of particular MHC alleles may influence the positive and negative selection of T lymphocytes during thymic maturation. Second, autoreactive lymphocytes that survive but are normally unresponsive to self antigens may be stimulated by cross-reactive antigens or by polyclonal activators that function independently of antigen receptor-mediated stimulation. Third, regulatory mechanisms that normally control the responses of all lymphocytes, including ones that are autoreactive, may be aberrant or nonfunctional. Immunologic studies of autoimmunity have focused on two broad aspects: (1) the stimuli that trigger the proliferation and effector functions of autoreactive lymphocytes, and (2) the nature of autoreactive B and T cells, in particular whether they are normally present cells that escape regulation or abnormal clones that are deleted or absent from healthy individuals.

Immunologic Cross-Reactions of Foreign and Self Antigens

One of the simplest experimental methods for inducing an autoimmune disease is to immunize an animal with a slightly altered form of a self antigen or with the homologous antigen from an animal of a different species. For example, rats and mice immunized with heat-denatured autologous thyroglobulin (a thyroid protein) or with rabbit thyroglobulin in adjuvant develop thyroiditis. Similarly, mice immunized with bovine or guinea pig myelin basic protein in adjuvant develop effector T cells that also recognize the mouse's own myelin basic protein and cause EAE. Thus, the immune response is induced by a foreign antigen or altered self antigen but the disease develops because the response is also directed against the homologous normal self antigen. Little is known about the mechanisms by which such immunizations with antigens that differ only slightly from self antigens lead to an apparent breakdown of self-tolerance. In the case of experimental autoimmune thyroiditis, it is possible that mice normally contain B cells capable of recognizing some epitope(s) of their own thyroglobulin; however, these B cells do not produce antibody because helper T cells specific for other determinants of the same protein are tolerant or have been deleted. If rabbit thyroglobulin has T cell epitopes that are different from mouse thyroglobulin, the rabbit protein injected into mice will activate helper T cells, which can cooperate with the self thyroglobulin-specific B cells (Fig. 18-7). This leads to stimulation of the B cells and the production of antibodies that bind to the mouse's own thyroglobulin and produce thyroiditis. Such immunologic cross-reactions can explain how autoantibodies might be produced against multideterminant self antigens.

Because autoimmune responses induced by immunologic cross-reactions are likely to generate autoantibodies specific for one or a few related antigens, it is likely that the lesions that develop are organ- or tissue-specific. Infections or inflammation secondary to trauma may lead to alterations in autologous proteins and generate autoimmunity by this mechanism. This may be the basis for the clinical observation that many organ-specific autoimmune diseases follow infectious prodromes or trauma.

Polyclonal Lymphocyte Activation

Autoimmunity can also result from antigen-independent stimulation of self-reactive clones that are not deleted during development. Polyclonal activators stimulate a large number of T or B lymphocytes irrespective of antigenic specificity and often without interacting with antigen receptors. The best example is lipopolysaccharide (LPS), which functions as a polyclonal B cell activator in mice. Mice injected with LPS produce antibodies of many specificities, included among which are autoantibodies. This may be because some self-reactive B cells are not deleted but rendered unresponsive (anergic) to self antigens (see Chapter 10). However, they retain the ability to proliferate and differentiate in response to stimuli such as LPS, which functions even in the absence of antigen receptor expression. *This form of autoimmunity, being a component of a polyclonal response, is usually associated with the production of multiple autoantibodies and therefore gives rise to systemic rather than organ-specific autoimmune diseases.* Systemic lupus erythematosus fits the criterion of a systemic autoimmune disease with multiple autoantibodies (see Box 18-1),

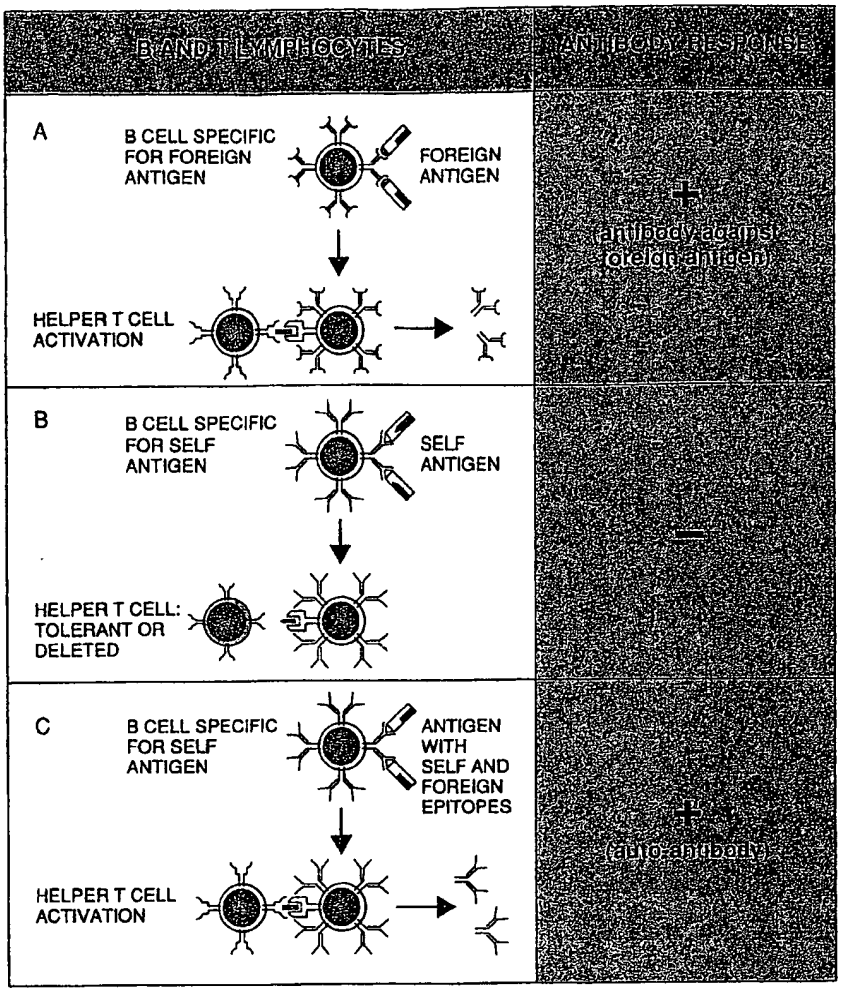


FIGURE 18-7. Role of helper T cells in the production of autoantibodies. *In a normal immune response* (A), *B and T lymphocytes specific for epitopes of a foreign antigen cooperate to stimulate antibody production. B cells specific for a self antigen may not be stimulated if helper T cells specific for the self antigen are absent or tolerant* (B). *However, these B cells may produce autoantibody if stimulated with an antigen containing foreign epitopes that are recognized by specific helper T cells* (C).

but the initiating agent for this disease is unknown. Polyclonal antibody secretion *in vivo* may be induced by microbial cell wall products similar to LPS, and this is another possible link between infections and autoimmunity. Multiple autoimmune phenomena are also associated with graft-versus-host disease (GVHD), which occasionally develops after the transplantation of allogeneic bone marrow (see Chapter 16). In recipients of such transplants, helper T cells may develop from precursors in the transplanted marrow and B cells may be derived from the host. If these two populations are allogeneic, the T cells recognize the host B cells as "foreign" and respond by secreting cytokines. This leads to polyclonal B cell activation and autoantibody production in the absence of specific antigenic stimulation.

Polyclonal T cell activation has also been postulated as a mechanism of autoimmunity. Adjuvants or bacterial "super-antigens" can stimulate large numbers of T cells, among which may be clones specific for but normally unresponsive to self antigens.

Abnormalities in Lymphocyte Activation and Regulation

A variety of immunoregulatory abnormalities has been associated with different autoimmune diseases, particularly in animal models. (NZB × NZW)F1 mice develop a disease resembling human SLE (see Box 18-1), and their B lymphocytes produce much higher amounts of antibodies in response to polyclonal activators and exogenous antigens than B cells from other strains that do not develop spontaneous autoimmunity. The biochemical basis of this B cell "hyperresponsiveness" is unknown. In contrast, MRL/*lpr* mice, which develop a SLE-like syndrome associated with

T cell proliferation, have normal B cell responses. It has been postulated that the spontaneously proliferating T cells in these mice constitutively produce a cytokine that stimulates B cell growth and differentiation. This cytokine appears distinct from the known B cell helper factors and has not yet been identified molecularly. It is important to distinguish between autoimmune disorders that may be caused by primary B cell abnormalities and those due to excessive cytokine production, because diagnostic and therapeutic approaches for these categories of diseases would be quite different even if their clinicopathologic manifestations were similar. The search for such immunologic aberrations in human autoimmune disorders has not yielded informative results to date.

It is also striking that many experimental autoimmune diseases, including thyroiditis and EAE, develop only if the antigens are administered with strong adjuvants. Such adjuvants may have two complementary effects. First, they may activate macrophages, which produce costimulators that function normally to overcome T cell anergy (see Chapter 10). Second, adjuvants may contain proteins, possibly resembling super-antigens, that are polyclonal activators of T cells.

Finally, various deficiencies in the numbers and/or function of suppressor T cells have been reported in many experimental and human autoimmune diseases. Attempts to isolate such suppressor cells have generally failed. There is, therefore, little direct evidence for a role of suppressor cells in the maintenance of self-tolerance and, conversely, for suppressor cell defects as a cause of autoimmunity.

The Nature of Autoreactive Lymphocytes: Analysis of Antigen Receptor V Genes in Autoimmunity

It is evident from our discussion so far that we know little about the specificity and development of autoreactive lymphocytes. One can postulate that the expression of particular antigen receptor variable (V) genes may contribute to autoimmunity in different ways. For instance, autoimmunity may be due to the presence in the germline of V genes that encode self antigen-specific receptors and that are absent in normal individuals. Individuals that contain these V genes may have an inherited propensity to develop autoimmunity. If, however, autoimmunity is due to a failure to delete self-reactive lymphocyte clones, then all individuals may contain the same germline V genes but the peripheral lymphocytes of individuals prone to develop these disorders may express antigen receptors that are absent from the normal selected repertoire of mature lymphocytes. It is also possible that autoimmunity is due to aberrant activation and regulation of lymphocytes that are present normally. In this case there may be no difference in V gene expression between autoimmune and normal individuals. Finally, autoreactive lymphocytes may arise as a consequence of abnormal somatic mutations involving antigen receptors that normally recognize foreign antigens, making these receptors reactive with self antigens.

One approach for evaluating these various possibilities is to compare the Ig and T cell receptor (TCR) V genes in the germlines of inbred strains of mice that do and do not develop spontaneous autoimmunity. In addition, one can determine if self-reactive lymphocytes or antibodies express V genes that are present in normal individuals or in lymphocytes or antibodies specific for foreign antigens. Such analyses are in their infancy and have not yet provided definitive answers to why autoimmunity develops. Nevertheless, the available data have already led to some significant conclusions:

1. *Autoimmunity is not due to a specific Ig or TCR repertoire and is not associated with a specific V gene polymorphism.* Comparisons of restriction fragment length polymorphisms (see Chapter 4, Box 4-1) in humans that do and do not have autoimmune diseases indicate no specific associations of the diseases with Ig gene loci. The Ig germline genes in two strains of mice that develop SLE-like syndromes, (NZB × NZW)F1 and MRL/*lpr* (see Box 18-1), also reveal no consistent differences from other, non-autoimmune strains. Autoantibodies of different specificities do not show similar patterns of V gene usage or somatic mutations. This argues against a common initiating antigen or stimulus for diverse autoimmune disorders. Limited analyses of TCR α and β loci have led to similar conclusions.

2. *Autoreactive B and T lymphocytes express essentially the same antigen receptor genes as do normal lymphocytes specific for foreign antigens.* For instance, anti-DNA antibody producing B cell hybridomas from (NZB × NZW)F1 mice, and rheumatoid factor producing hybridomas from MRL/*lpr* mice, contain Ig V, D, and J gene segments that are also found in various combinations in hybridomas that secrete antibodies specific for foreign antigens. The Ig genes of rheumatoid factor-producing B cells also show somatic mutations in V regions that suggest that these autoantibodies are produced by the same types of antigenic stimulation and lymphocyte selection that are operative in humoral immune responses to foreign antigens. Interestingly, in mice, the small subset of B cells that expresses CD5 accounts for a disproportionately high level of autoantibody production. These cells secrete IgM autoantibodies against a variety of self antigens spontaneously in culture, i.e., without overt antigenic stimulation. The significance of $CD5^+$ B cells in human autoimmune diseases is not clear.

3. *In individual patients or inbred mouse strains with organ-specific autoimmune diseases, the autoreactive lymphocytes may be oligoclonal or may express restricted sets of V genes.* In an oligoclonal population, which is derived from a few precursors, only a small number of antigen receptor genes is expressed. Restricted V gene expression may, however, also be seen in a lymphocyte population that is multiclonal in origin, and this suggests that the population is specific

for one or a few antigenic determinants. For instance, the majority of the T cell clones isolated from the synovium of an individual patient with rheumatoid arthritis express the same germline TCR genes, indicating that they arose from the same T cell. Rheumatoid factor-producing B cells from individual MRL/*lpr* mice are similarly oligoclonal. T cells cloned from the cerebrospinal fluid of individual patients with multiple sclerosis also express only a few V genes. However, different patients or inbred mouse strains with the same disease may express different Ig or TCR V genes in their self-reactive lymphocytes. Therefore, the restricted V gene expression in autoreactive lymphocytes in individuals probably reflects the fact that these cells are specific for one or few antigenic determinants rather than a consistent association between a particular pattern of V gene usage and the development of autoimmunity. Alternatively, if only a few autoreactive cells escape tolerance induction or regulation at any given time, the cells isolated from lesions would be oligoclonal.

The general conclusion of these studies is that *organ-specific autoimmune diseases are usually due to stimulation of lymphocyte clones having a limited repertoire.* The molecular characteristics of many autoreactive antibodies suggest that their production is essentially similar to the generation of antibodies specific for foreign protein antigens. These studies do not tell us whether autoreactive clones are abnormal cells that have escaped the process of self-tolerance or are normally present cells whose aberrant stimulation or regulation leads to pathologic autoimmunity. They also do not indicate whether the primary lesion leading to autoantibody production is in the B cells or in helper T cells or both.

GENETIC FACTORS IN AUTOIMMUNITY

From the earliest studies of patients with autoimmune disorders, it has been known that some of these diseases run in families and there is a high rate of concordance in monozygotic twins. Much of the interest in the genetic basis of autoimmunity has focused on MHC genes, because of their known role in the selection of T cells and in the induction of immune responses to protein antigens. Although it is not yet clear precisely how MHC genes influence the development of autoimmunity, recent molecular and biochemical studies are providing new insights into mechanisms as well as potential therapeutic approaches.

Role of MHC Genes in Autoimmunity

Human leukocyte antigen (HLA) typing of large groups of patients with various autoimmune diseases has shown that some HLA alleles occur at higher frequencies in patients with particular diseases than in the general population. From such studies, it is possible to estimate the "relative risk" of developing a disease with every known HLA allele (Table 18-6). The strongest such association is between ankylosing spondylitis, an autoimmune disease of vertebral joints, and the class I MHC allele, HLA-B27. The immunologic basis of this is not well understood. Recently, much more work has been done on the polymorphic class II alleles, HLA-DR and DQ, in autoimmune diseases, because of the realization that class II MHC molecules are crucial for the selection and activation of $CD4^+$ T cells and, therefore, for the regulation of all immune responses to protein antigens.

TABLE 18-6. Examples of Human Leukocyte Antigen (HLA)-Linked Immunologic Diseases

Disease	HLA Allele	Relative Risk*
Rheumatoid arthritis	DR4	6
Insulin-dependent diabetes mellitus	DR3	5
	DR4	6-7
	DR3/DR4	20
	DR3, DQw8	100
	DR2	0.25
Pemphigus vulgaris	DR4	24
Chronic active hepatitis	DR3	14
Sjögren's syndrome	DR3	10
Celiac disease	DR3	12
Ankylosing spondylitis	B27	90

* Relative risk defines the chance of individuals with a particular HLA allele(s) of developing a disease compared with individuals lacking that HLA allele(s).

DNA sequencing of class II MHC genes, combined with analysis of restriction fragment length polymorphisms to identify small genetic differences between patients and controls, have clearly established the association of particular class II MHC sequences with some autoimmune diseases. Two examples are especially illustrative:

1. *Insulin-dependent diabetes mellitus* (Box 18-3) is both positively and negatively associated with HLA genes. Ninety to 95 per cent of Caucasians with this disease have HLA-DR3 or HLA-DR4 or both, in contrast to about 40 per cent of normals, and 40 to 50 per cent of patients are HLA-DR3/DR4 heterozygotes, in contrast to 5 per cent of normals. It is now apparent that the HLA-associated susceptibility to IDDM is more strongly linked to particular HLA-DQ alleles, which are in linkage disequilibrium (i.e., inherited together) with certain HLA-DR alleles. All DQβ chains that are more frequent in Caucasian patients than in controls have one of three amino acids (alanine, valine, or serine) at position 57, whereas the DQβ chains present at lower frequency in patients than in controls have aspartic acid (Asp) at this position. Interestingly, the non-obese diabetic (NOD) mouse, which develops a spontaneous IDDM-like disease, has serine at position 57 of I-Aβ, the murine homolog of DQβ. In contrast, most other mouse strains, including the

closely related non-obese normal, have Asp at this position. These observations led to the hypothesis that Asp 57 in the DQβ chain confers resistance to IDDM and its absence increases susceptibility. This residue forms a part of the antigen-binding cleft of the class II MHC molecule (see Chapter 5), supporting the idea that it influences T cell selection or antigen recognition. There are, however, several exceptions to this association of Asp 57 with IDDM. For instance, Japanese patients with the disease frequently have Asp 57 in their DQβ chains. Moreover, if a transgenic I-A molecule containing serine at position 57 in the β chain is expressed in NOD mice, it too reduces the incidence of IDDM. *It is, therefore, likely that the structure of the peptide-binding cleft of the entire MHC molecule contributes to the development of this autoimmune disease,* and residue 57 may be one of many amino acids that play a role in determining this structure. Furthermore, the HLA linkage of IDDM is unusual, in that it maps as a recessive trait. The likely reason for this is that increased susceptibility is associated with the absence of certain residues in the DQβ chain of both alleles, and these residues in either allele are significantly protective.

2. *Rheumatoid arthritis* (see Box 18-2) is most strongly associated with HLA-DR4 and less so with the DR1 and DRw10 haplotypes. In all these alleles, the amino acid sequences from positions 65 to 75 of the DRβ chain are almost identical. These sequences are located in a polymorphic region of the class II MHC molecule that also participates in the formation of the antigen-binding cleft.

There are several postulated mechanisms to account for the association of autoimmune diseases with class II MHC sequences.

1. The amino acid sequences of MHC molecules in an individual may determine which clones of potentially autoreactive T cells are positively selected or deleted during development. As we discussed in Chapter 8, the expression of particular MHC genes is crucial during thymic education, because T cells recognize peptides attached to the antigen-binding clefts of MHC molecules and this recognition determines the selection of developing T lymphocytes. Thus, if the MHC molecules in the thymus of an individual fail to bind a self protein with high affinity, T cells reactive with this self antigen may escape clonal deletion. Such a mechanism might explain the HLA-DQβ-associated resistance to IDDM. If this MHC molecule is capable of binding and presenting the self antigen(s) responsible for insulitis, the presence of this allele results in deletion or inactivation of T cells specific for this self antigen. In the absence of this MHC allele, the autoreactive T cells might escape negative selection and may later cause insulitis. Presumably, these T cells are restricted to recognizing the relevant self antigen in association with another HLA molecule, which, for unknown reasons, does not negatively select the cells during their maturation.

2. Similarities between MHC molecules and self or microbial antigens may contribute to autoimmunity. For example, if a microbial antigen resembles a self MHC molecule, T cell responses against the microbe may result in cross-reactions against self MHC. This is an example of molecular mimicry. Several bacterial and viral proteins contain stretches of amino acids that are also found in various MHC molecules, but the pathogenic significance of these findings is uncertain.

3. Class II MHC molecules may influence the activation of T cells such as suppressor cells, whose function is to prevent autoimmune reactions. However, as stated above, the role of regulatory T cells in self-tolerance is not clearly established.

4. An older hypothesis was that HLA-disease associations reflected the ability of some but not other MHC molecules to bind self antigens and present these antigens to specific T cells. According to this postulate, autoreactive T cells were neither deleted nor rendered anergic. Instead, they were unable to respond to self antigens because normal individuals could not present these antigens in association with their own MHC molecules. However, we now know that MHC molecules normally bind many, and perhaps all, self peptides and do not discriminate between self and non-self at the stage of antigen presentation (see Chapter 6). Therefore, this hypothesis is unlikely to be correct.

Finally, we should add that disease-associated HLA sequences are found in healthy individuals and, conversely, that alleles commonly present in normal individuals are also found in some patients with autoimmune diseases. *Therefore, the expression of a particular HLA gene is not by itself the cause of any autoimmune disease* but is likely to be one of several factors that contribute to autoimmunity. It is also clear that HLA-disease associations are often weak or partial. In some cases, this may be because an HLA "allele" identified by tissue typing is actually a family of structurally related alleles, some of which have strong disease associations and others that do not.

The concept of HLA-linked autoimmunity does have practical implications. It has some predictive value, the usefulness of which depends on the strength of the disease association. It may also be possible to exploit this concept for specific immunotherapy. For instance, if a polymorphic MHC sequence is associated with a disease in a patient, antibodies against this sequence or peptides that bind to this site in the MHC but do not stimulate T cells may block T cell antigen recognition and alter the course of the disease. This idea has been successfully used to prevent EAE in inbred mice (Box 18-4). Whether or not such approaches will be feasible in the clinical setting remains to be seen.

Association of Other Genes with Autoimmunity

The development of autoimmunity is clearly influenced by multiple genes. Breeding analyses of

mouse strains that develop autoimmunity indicate that as many as twenty genes may contribute to different diseases. Even in autoimmune diseases that are associated with class I MHC (HLA-A or HLA-B) alleles, it is thought that the apparent association reflects a linkage disequilibrium with another adjacent gene(s) that is actually responsible for disease susceptibility. Some of these non-MHC genes may influence immune reactions and others may cause disease by non-immune mechanisms. In humans, genes for two complement proteins, C2 and C4, and for two cytokines, tumor necrosis factor (TNF) and lymphotoxin (LT), are located within the MHC locus (see Chapter 5). It is known that C2 and C4 deficiencies lead to impaired phagocytosis of immune complexes and an increased incidence of SLE-like syndromes. In addition, certain alleles of C2 and C4 genes may be in linkage disequilibrium with MHC alleles that predispose to autoimmunity. Recent data suggest that in humans TNF genes are also polymorphic, and some alleles may be associated with autoimmune diseases. Such findings have led to the concept of "extended HLA haplotypes." In some HLA haplotypes, recombination within the locus appears to be suppressed, so that many genes (both HLA genes and other adjacent genes) remain in linkage disequilibrium. We do not know the molecular mechanism or evolutionary advantage of maintaining an extended haplotype in linkage disequilibrium, but some such extended haplotypes are present with increased frequency in patients with certain autoimmune diseases.

In the final analysis, it is clear that genes presently identified do not completely account for the inheritance patterns of autoimmune diseases. However, because of the remarkable progress that has been made in the late 1980s in techniques for identifying disease-producing genes and mapping the human genome, it is likely that the genetic basis of autoimmunity will be understood in increasingly precise terms in the not too distant future.

Other Factors in Autoimmunity

The development of autoimmunity is also related to a number of other factors:

1. *Anatomic alterations* may lead to the exposure of antigens that are normally sequestered and concealed from the immune system. Because of this sequestration, individuals may not be immunologically tolerant to such antigens. Therefore, if such self antigens are released and interact with immunocompetent lymphocytes, specific immune responses may develop. Examples of anatomically sequestered antigens may be intraocular proteins and sperm. Post-traumatic uveitis and orchitis, and orchitis following vasectomy, are thought to be autoimmune responses to self antigens that are released from their normal locations.

2. *Hormonal influences* are also thought to play a role in human and experimental autoimmune disorders. SLE, for instance, affects females about ten times as frequently as males. The lupus-like disease of (NZB × NZW)F1 mice also develops in females and can be retarded by androgen treatment. Many other autoimmune disorders tend to be more frequent in females, although this is clearly not always the case. It is not known whether this is due to the influence of sex hormones or other factors.

3. *Viral and bacterial infections,* as mentioned in the preceding sections, are associated with autoimmunity, and infections often precede the clinical manifestations of autoimmune diseases. In most of these diseases, the infectious microorganism is not present in autoimmune lesions and is not even detectable when autoimmunity develops. Therefore, the lesions are not due to the infectious agent itself but result from host immune responses. The many possible effects of infections include polyclonal lymphocyte activation, alterations of self antigens to create partially cross-reactive neo-antigens, mimicry of self antigens, and tissue injury leading to release of anatomically sequestered antigens.

It is appropriate to conclude by reminding ourselves that "mechanisms of autoimmunity" is a topic in which theories and hypotheses continue to outnumber facts. However, we now know a great deal about the immunologic basis of self/non-self discrimination and the induction and maintenance of self-tolerance, and remarkable technical advances have been made in analyzing genetic polymorphisms and structural variations in Ig, TCR, and MHC genes. There seems little doubt that as these new concepts and methods are applied to human and experimental autoimmune diseases, clearer and more definitive answers to the enigmas of autoimmunity will begin to emerge.

Summary

Diseases in which tissue injury and pathophysiologic abnormalities are due to immunologic mechanisms may be initiated by immune responses to foreign or self (autologous) antigens. Pathogenic mechanisms include antigen-antibody complexes formed during humoral immune responses, autoantibodies against fixed tissue or cell surface antigens, and T lymphocytes. The effector mechanisms by which antibodies and immune complexes induce tissue injury include the complement system and various host inflammatory cells. Antibodies against physiologic agents such as hormones or against cell surface receptors for hormones induce functional abnormalities without the involvement of any other effector systems. $CD4^+$ T lymphocytes recruit and activate macrophages as the principal effectors of tissue injury, and $CD8^+$ cytolytic T lymphocytes themselves lyse antigen-bearing target cells.

Immune responses against foreign antigens may be pathologic either because of immunologic cross-reactivity with self antigens or because the responses are excessive or unregulated (hence the term "hyper-

sensitivity" applied to such reactions). Strong immune responses to self antigens, called autoimmunity, are usually pathologic, because normal individuals are tolerant to self antigens. Autoimmune responses develop as a result of multiple interacting factors. The principal immunologic mechanisms that may contribute to autoimmunity include polyclonal lymphocyte stimulation, the introduction of foreign antigens that are partially cross-reactive with self molecules, and abnormalities in immunoregulation. The strongest genetic association of autoimmunity is with MHC genes, and multiple mechanisms have been proposed to account for such associations. Recent advances in the understanding of self-tolerance and self/non-self discrimination and in techniques for analyzing Ig, TCR, and MHC genes hold great promise for elucidating the mechanisms of autoimmunity and for developing rational therapeutic strategies for this group of diseases.

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